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Decontamination Options for Sensitive Equipment-related Materials Contaminated with a Fourth Generation Agent (FGA)

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DISCLAIMER

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FOREWORD

The U.S. Environmental Protection Agency (EPA) is charged by Congress with protecting the Nation's land, air, and water resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, EPA's research program is providing data and technical support for solving environmental problems today and building a science knowledge base necessary to manage our ecological resources wisely, understand how pollutants affect our health, and prevent or reduce environmental risks in the future.

The Center for Environmental Solutions and Emergency Response (CESER) within the Office of Research and Development (ORD) conducts applied, stakeholder-driven research and provides responsive technical support to help solve the Nation's environmental challenges. The Center's research focuses on innovative approaches to address environmental challenges associated with the built environment. We develop technologies and decision-support tools to help safeguard public water systems and groundwater, guide sustainable materials management, remediate sites from traditional contamination sources and emerging environmental stressors, and address potential threats from terrorism and natural disasters. CESER collaborates with both public and private sector partners to foster technologies that improve the effectiveness and reduce the cost of compliance, while anticipating emerging problems. We provide technical support to EPA regions and programs, states, tribal nations, and federal partners, and serve as the interagency liaison for EPA in homeland security research and technology. The Center is a leader in providing scientific solutions to protect human health and the environment.

This report provides information on decontamination options for surfaces contaminated with a fourth generation (chemical) agent. Decontaminants and surfaces were selected based on expected higher material compatibility than hypochlorite-based oxidants (e.g., corrosive bleach) with materials associated with sensitive equipment.

Gregory Sales, Director

Center for Environmental Solutions and Emergency Response

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This effort was directed by the Task Order Contracting Officer Representative (TOCOR) from the Office of Research and Development's (ORD's) Homeland Security and Materials Management Division (HSMMD) within the Center for Environmental Solutions and Emergency Response (CESER). The contributions of the following individuals have been a valued asset throughout this effort.

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EXECUTIVE SUMMARY

The U.S. Environmental Protection Agency (EPA) is designated as a coordinating Agency, under the National Response Framework, to prepare for, respond to, and recover from a threat to public health, welfare, or the environment caused by actual or potential oil and hazardous materials incidents. The imminent threat of a chemical agent release is driving EPA's Homeland Security Research Program (HSRP) to develop a research program that systematically evaluates potential decontamination technologies for chemical agents. A new class of chemicals, known as fourth generation agents (FGAs), has emerged as a new homeland security threat. Like chemical warfare agent (CWA) nerve agents, FGA compounds bind to acetylcholinesterase receptor sites leading to rapid muscle twitching, seizures, and potential death when inhaled, consumed, or exposed to skin. FGAs are persistent under certain environmental conditions and are considered more toxic than traditional CWAs, requiring an even higher scrutiny to remediate contaminated sites. In 2019, three FGAs (A-230, A-232, and A-234) were added to the Organisation for the Prohibition of Chemical Weapons (OPCW) list of Schedule 1 CWAs.

EPA has conducted prior decontamination research for traditional CWAs, but there is a scientific data gap for decontamination technologies capable of remediating FGA-contaminated sensitive equipment (SE). The purpose of this project was to evaluate the efficacy of commercial off the shelf (COTS) hydrogen peroxide-based and/or peroxyacetic acid-based decontamination technologies for decontamination of one of the FGAs, A-234, on sensitive equipment materials.

The decontaminants that were investigated in this study include Dahlgren Decon™, Decon PLUS™, and EasyDECON® DF200. All the decontaminants use a peroxy species for oxidation and surfactant(s) to enhance transport of the oxidant to the contaminant. These types of decontaminants are generally considered less corrosive than, e.g., hypochlorite-based oxidants and hence have been proposed to have increased compatibility with SE, which may fail if excessively corroded. Dahlgren Decon™ and Decon PLUS™ both use activated peroxyacetic acid technologies. EasyDECON® DF200 uses a hydrogen peroxide-based technology with an activator which leads to a peroxy acid. The four SE materials selected for this study were acrylonitrile butadiene styrene (ABS), silicone, Gorilla Glass®, and high-impact polystyrene (HIPS). These materials are frequently encountered in protective housings for electronics, seals, gaskets, keyboards, and detector equipment. In addition, two types of sensitive equipment proxies (water-resistant calculators and iPhones) were also included in the study. A-234 was applied as liquid droplets to achieve a target contamination density of 2 g/m² to the surface of test coupons having a surface area of 10 cm², test panels having a surface area of 302 cm², and full SE items.

Decontaminants were applied using a semi-automated spray system at a target application volume of 60 to 100 µL/cm². This application rate aligns with decontamination application rates using backpack sprayers in a full-scale field study. Following the specified decontaminant dwell periods, the test coupons, wipes and/or decontaminant overspray/rinsate were extracted in organic solvent and analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) to quantify the mass of A-234 remaining in the extracts.

Results

- The measured decontamination efficacies for EasyDECON® DF200 and Decon PLUS™ with small coupons of the four SE materials ranged between 99.4% and >99.99%

(EasyDECON DF200) and between 99.0% and >99.99% (Decon PLUS™) (refer to Figure ES-1). ANOVA statistical analysis demonstrated that the mean residual A-234 mass on the silicone material for both EasyDECON® DF200 and Decon PLUS™ was significantly higher than on the other three materials. This could be important to responders in the field if they need to decontaminate equipment containing higher amounts of silicone material. Analysis of the quenched rinsate samples revealed that between 0.04% and 0.003% of the initial A-234 challenge (based on positive controls) remained in the immediate rinsate for the EasyDECON® DF200 and between 0.02% and 0.003% for the Decon PLUS™. All rinsate samples quenched after 24-hr resulted in A-234 masses that were below detection limits.

- The measured decontamination efficacies for the Dahlgren Decon™ ranged from 91.5% to 99.3% for the four material types. Analysis of the rinsate samples revealed that between 63% and 101% of the initial A-234 challenge (based on positive controls) remained in the rinsate. These results suggest that the Dahlgren Decon™ may be effective at physically removing A-234 from SE surfaces, but it will likely present challenges to full-scale operational remediation efforts because of potential hazards associated with significant amounts of residual A-234 remaining in the decontamination rinsate, if these rinsates are not appropriately managed. The lower reactivity of Dahlgren Decon™ with A-234 is not novel, and reactivity with other FGAs may be different.
- The studies using water only as a decontaminant (i.e., no active chemical decontaminants) showed decontamination efficacies ranging from 96.0% to 98.9% across the four materials. Analysis of the rinsate samples showed that between 53% to 93% of the initial A-234 challenge (based on positive controls) remained in the rinsate. These results suggest that a water rinse may be effective at removing A-234 from these SE surfaces, but it will likely present challenges to full-scale operational remediation efforts because of potential hazards associated with residual A-234 remaining in the decontamination rinsate, if these rinsates are not appropriately managed.
- The efficacy on larger HIPS and silicone panels ranged between 97.5% and >99.99% (EasyDECON DF200) and between 94.3% and >99.99% (Decon PLUS™) based on the specific procedure modifications made to accommodate testing for the panels (refer to Figure ES-2). ANOVA statistical tests indicated that the mean residual A-234 mass remaining on the HIPS and silicone panels was statistically lower for the EasyDECON® DF200 compared to the Decon PLUS™.
- Increasing the decontaminant dwell time from 60 to 120 min did not have a statistically significant impact on efficacy. The decontamination efficacy for the 60-min dwell time of the Decon PLUS™ ranged from 99.4% to > 99.99%, while the 120-min dwell time resulted in decontamination efficacy ranging between 99.2% and 99.95% for the four SE materials.
- Adding a blotting step (which physically removes the contaminant via an absorbent material) to the sampling procedure post-decontamination increased the efficacy for the Decon PLUS™ procedure. Based on statistical analysis of the test data, there was a significant difference in performance for the Decon PLUS™ (95.0% to 99.5%) by adding the blotting step prior to the post-decontamination wipe sampling process. For the

EasyDECON® DF200, there was no statistical difference in efficacy (99.2% to 99.98%) from the addition of the blotting step.

- Based on statistical analysis of the test data, increasing the number of decontaminant spray applications from one to two resulted in a measurable increase in decontamination efficacy for the EasyDECON® DF200 (99.98% to > 99.99%) procedure on the HIPS material. For the Decon PLUS™, there was no statistical difference in efficacy (99.94% to >99.99%) for an additional decontaminant spray application on the HIPS material. For the tests using two decontaminant spray applications, the amounts of A-234 in the replicate samples for both EasyDECON® DF200 and Decon PLUS™ were all below method detection limits.
- Evaluation of SE proxy items (calculators and iPhones) using EasyDECON® DF200 and Decon PLUS™ had a final set of parameters comprising two sequential decontaminant applications (each with a 60-min dwell time), blotting and wipe sampling. Based on statistical analysis of the test data, there was no significant difference in performance between EasyDECON® DF200 (99.8% and > 99.99% efficacy on the calculators and iPhones, respectively) and Decon PLUS™ (99.6% and 99.5% efficacy on the calculators and iPhones, respectively) (refer to Figure ES-2). The SE item tests demonstrated that the wipe sampling is likely not an effective method to collect FGA from complex surfaces as demonstrated by the inability to collect FGA from the calculator button recesses of the positive control samples. These recovery results were between 15% to 32% of the initial contamination that was collected on the wipe sampler (compared to 79% to 91% of the initial contamination mass collected for the positive controls contaminated on the tops of the buttons). As such, caution should be employed by first responders when using this method to analyze complex surfaces for residual chemicals, and alternate methodologies should be developed and deployed in an operational environment.

Figure ES-1 summarizes the average percent decontamination efficacies measured for each test condition. The material-specific decontamination efficacies were consistent within each study, with silicone consistently showing a slightly lower efficacy than ABS, Gorilla Glass, and HIPS. Overall, the results show that decontamination efficiencies corresponding to almost complete A-234 destruction can be achieved in some (but not all) circumstances, particularly with the EasyDECON® DF200 and Decon PLUS™ products. Figure ES-2 summarizes the average percent decontamination for the large panel tests and the SE proxy items.

The study did not include analysis for decontamination degradation or breakdown products which may be toxic. Such analysis was outside the scope of this FGA decontamination efficacy study but should be considered as part of future efforts.

The results of this study will assist EPA responders, local and state governments, and health departments in the selection of decontamination products and in the development of procedures and methods for the decontamination of A-234 from sensitive equipment in the field.

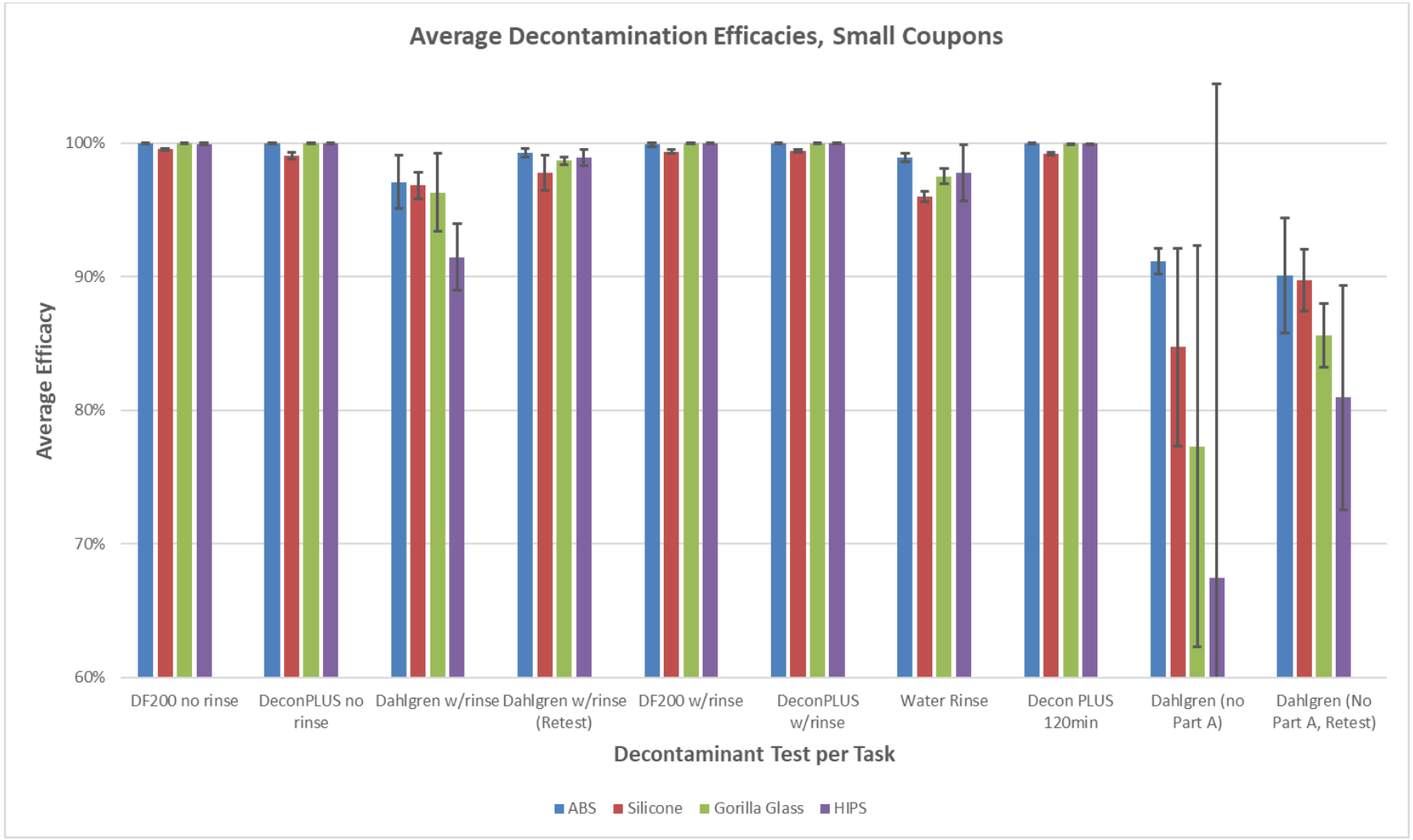

Figure ES-1. Average Decontamination Efficacies, Small Size Coupons

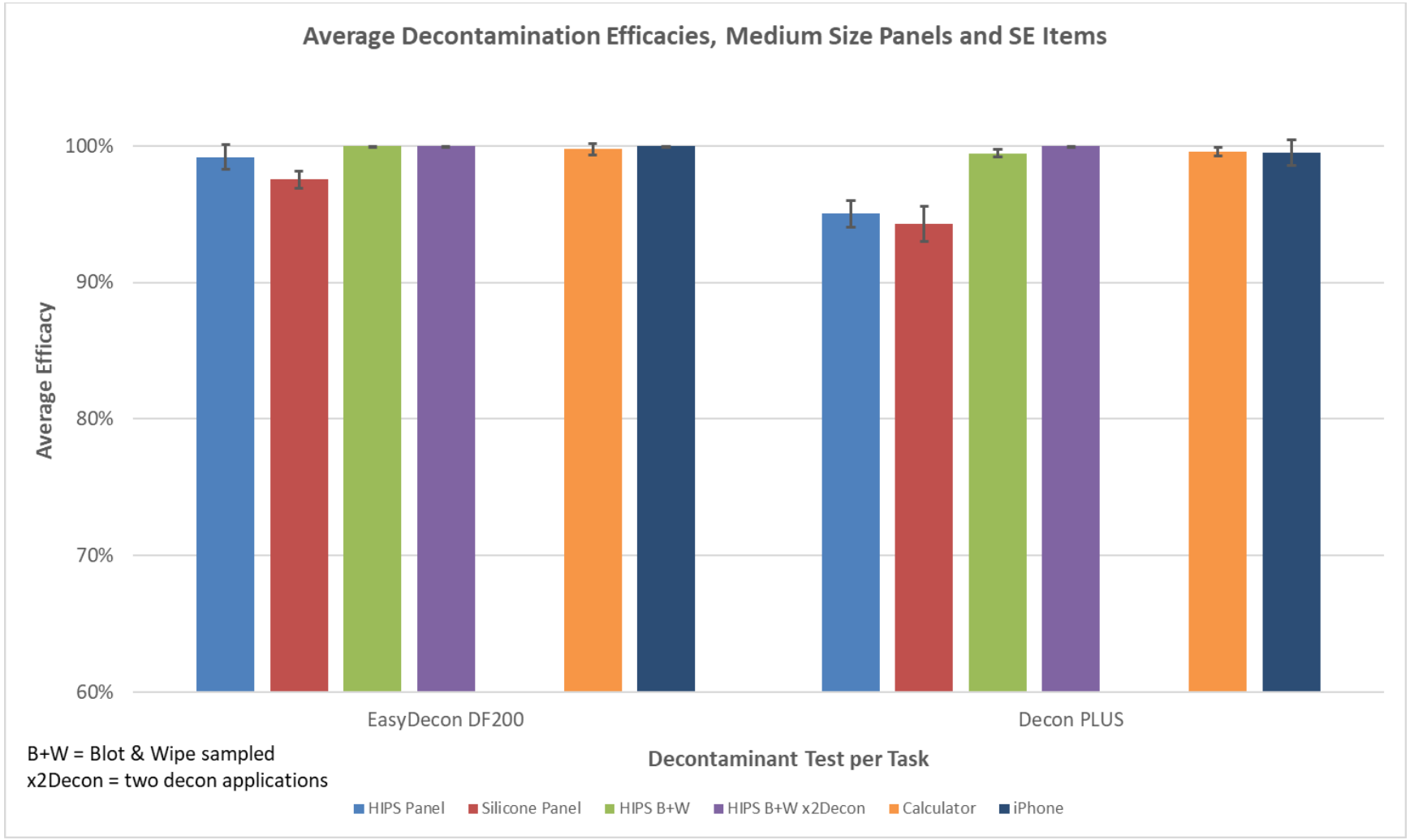

Figure ES-2. Average Decontamination Efficacies, Medium Size Panels and SE Items

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LIST OF ACRONYMS AND ABBREVIATIONS

°C	Celsius
µL	microliter(s)
ABS	Acrylonitrile Butadiene Styrene
ACTF	Avarint Chemical Test Facility
CAS	Chemical Abstracts Service
CBRN	Chemical, Biological, Radiological, and Nuclear
CCV	Continuous Calibration Verification
CESER	Center for Environmental Solutions and Emergency Response
cm	centimeter(s)
cm^2	square centimeter(s)
CMAD	Consequence Management Advisory Division
CNC	Computer Numerical Control
COTS	commercial off the shelf
CWA	Chemical Warfare Agent
DEMTMP	Diethyl (methylthiomethyl) phosphonate
DI	de-ionized (-water)
DSS	Decontaminant Spray System
EI	electron ionization
EPA	U.S. Environmental Protection Agency
ERG	Eastern Research Group, Inc.
FGA	Fourth Generation Agent
fl oz	fluid ounce(s)
g	gram(s)
GA	Tabun nerve agent
GB	Sarin nerve agent
GC-MS	Gas Chromatography Mass Spectrometer
GD	Soman nerve agent
HIE	High Ion Exchange
HIPS	High Impact Polystyrene
HSMMD	Homeland Security and Material Management Division
hr	hour(s)
HSRP	Homeland Security Research Program
IPA	isopropanol
IS	Internal Standard
kHz	kilohertz
L	Liter(s)
LC	Liquid Chromatography
LC-MS/MS	Liquid Chromatography Tandem Mass Spectrometer/Spectrometry
m	meter(s)
M	Molar
mg	milligram(s)
min	minute(s)
mL	milliliter(s)
mm	millimeter(s)
MRL	Method Response Limit

MRM	Multiple Reaction Monitoring
m/z	mass to charge ratio
ng	nanogram(s)
NIST	National Institute of Standards and Technology
OEM	Office of Emergency Management (US EPA)
OLEM	Office of Land and Emergency Management (US EPA)
OPCW	Organisation for the Prohibition of Chemical Weapons
ORD	Office of Research and Development (US EPA)
P	primary
PN	part number
ppt	part(s) per trillion
QA	Quality Assurance
QC	Quality Control
R^2	correlation coefficient
RH	Relative Humidity
RSD	relative standard deviation
S	secondary
SE	sensitive equipment
SS	Stainless steel
STS	sodium thiosulfate
THT	Tetrahydrothiophene
TOCOR	Task Order Contracting Officer Representative
UHPLC	Ultra-High Performance Liquid Chromatography
V	Volt

INTRODUCTION

The U.S. Environmental Protection Agency (EPA) is designated as a coordinating Agency, under the National Response Framework, to prepare for, respond to, and recover from threats to public health, welfare, or the environment caused by actual or potential hazardous materials incidents. Hazardous materials include chemical, biological, and radiological substances, whether accidentally or intentionally released. The imminent threat of a chemical agent release is driving EPA's Homeland Security Research Program (HSRP) to develop a research program that systematically evaluates potential decontamination technologies for chemical agents. EPA may be tasked to clean up contaminated areas.

Of concern to EPA are traditional chemical warfare agents (CWAs) including low-persistence G-series agents such as Tabun (GA), Sarin (GB), and Soman (GD), and high-persistence V-series agents such as VX. Their environmental persistence drives the need to actively remediate contaminated sites. A newer class of CWAs, known as Fourth Generation Agents (FGAs), have emerged as a new homeland security threat. Like traditional CWA nerve agents, FGA compounds bind to acetylcholinesterase receptor sites, leading to rapid muscle twitching, seizures, and potential death when inhaled, consumed, or exposed to skin. FGAs are persistent under certain environmental conditions and are considered more toxic than some traditional CWAs, so they require even higher scrutiny to remediate contaminated sites.

In March 2018, the poisoning of two individuals in Salisbury, UK using an FGA (also known as the "Novichok" family of agents) led to multiple contaminated sites across Salisbury and surrounding areas [1]. In June 2018, a second accidental exposure to the same agent in Amesbury, UK led to one fatality and additional contaminated sites. The remediation of both incidents was not completed until just under one year later (March 1, 2019) [2]. A similar scenario in a larger city or at a larger scale might have led to increased fatalities and injuries and a significant impact on the local economy. As is clear from this incident, the initial remediation strategy was hampered by a lack of knowledge on how to sample, analyze, decontaminate, and conduct waste management of the contaminated areas. This has led to a resurgence in the identification of research gaps related to the remediation of FGA-contaminated sites. One identified gap is a lack of knowledge with respect to effectively decontaminating sensitive equipment (SE) used by first responders, including high-cost equipment and/or instrumentation considered part of the critical infrastructure. SE-related materials are the materials used to fabricate sensitive equipment and as such, should not be impacted, damaged, or lose critical characteristics following contact with decontamination solutions that are used to degrade and/or remove a CWA on the material's surface. Otherwise, decontamination approaches for sensitive equipment could degrade the equipment's ability to operate at a defined level of functionality.

CWA decontaminants using chlorine chemistry (e.g., hypochlorite bleach) are known to be corrosive to many materials of construction so have a high probability of negatively impacting the functional performance of sensitive equipment. Alternative decontaminants against traditional persistent CWAs that may have less impact on sensitive equipment were identified in a previous literature study, with three options evaluated for efficacy [3]. Two of these decontaminants were Dahlgren Decon™ and EasyDECON® DF200, which both utilize peroxy chemistries which can be less corrosive than chlorine-based oxidants. The efficacy of those or other related, peroxy-based decontamination products against an FGA on SE-related material surfaces is unknown.

1.1 Purpose

The purpose of this project was to investigate the efficacy of several commercially available decontamination products for SE-related materials contaminated with an FGA and, if necessary, optimize the decontamination approaches to improve efficacy. The technical approach included the following key elements:

- Use of a backpack sprayer nozzle integrated into a semi-automated decontamination system to closely replicate application rates of decontaminants in the field.
- Inclusion of tests to span the breadth of possible real-world field scenarios and to ensure that residual decontaminant is removed from surfaces to mitigate potential analytical interferences of post-decontamination samples.
- Evaluation of quenching techniques for the select decontaminants and analysis procedures to ensure compliance with desired quality assurance (QA)/quality control (QC) criteria.
- Determination of efficacy through the analysis of post-decontamination residual FGA, not including degradation or breakdown products.

1.2 Project Objectives

Specific objectives of this study were to:

- Determine whether the commercially available peroxyacetic acid and other peroxy-based decontamination technologies that have shown good efficacy against traditional CWAs are also capable of effectively decontaminating FGA on SE surfaces.
- Evaluate decontamination efficacy on multiple material types using small coupons, large panels, and SE items.
- Implement procedural changes (e.g., water rinse, longer decontaminant dwell times, re-application of decontaminant) and measure changes in decontamination efficacies resulting from these changes.

1.3 Test Facility Description

All tests were conducted at Avarint's Chemical Test Facility (ACTF), which is located on a remote 700-acre site approximately 35 miles south of Buffalo, New York. The ACTF operates under a Provisioning Agreement with the U.S. Army Materiel Command that authorizes Avarint to receive, store and use chemical warfare agents in support of U.S. Government programs. All activities at the site are conducted in strict compliance with the terms of the Provisioning Agreement and all other federal, state, and local regulations.

EXPERIMENTAL METHODS

2.1 Experimental Design

Decontamination efficacy was evaluated through a series of surface decontamination tests. The testing was performed using the FGA A-234. Three (3) commercially available decontaminants were evaluated (refer to Section 2.3.9) to determine the decontamination efficacy of each technology against this FGA from the surface of coupons or panels of four (4) materials often found in equipment used by first responders. Testing was also performed with two (2) SE proxy items to determine whether the small coupon and panel test results are indicative of larger-scale performance in the field. Prior to the execution of the surface decontamination tests, the test methods were experimentally demonstrated, and the results were evaluated against predefined quality assurance criteria.

The project tasks were structured to begin the decontamination efficacy testing at a small scale and systematically increase the complexity and scale of the experiments to improve performance and demonstrate operational relevance. Interim results generated throughout the project were used to inform method modifications for subsequent testing tasks.

2.2 Test Methods

2.2.1 Method Demonstration/Development

A series of method demonstration/development studies (see Table 1) were performed to:

- Determine the extraction efficiency of A-234 from the four materials tested.
- Determine the extraction efficiency of A-234 from sampling wipes (wipes were used for the large panel and full SE item tests).
- Develop effective quench methods to neutralize decontaminants within rinsate/runoff samples and coupon and wipe solvent extract samples.

Each study is described in more detail in the following sections.

Table 1. Test Matrix for Pre-Studies

Study Name	Materials	Solvent	No. of Dosing Levels	Decontaminants	Quench
Coupon Extraction Efficiency	ABS, silicone, Gorilla Glass®, HIPS	IPA	4	None	No
Wipe Extraction Efficiency	Wipe Sample	IPA	4	None	No
Coupon Quench	Stainless Steel	IPA	1	Dahlgren Decon™, EasyDECON® DF200, Decon PLUS™	No
	Stainless Steel	IPA	1		Yes
Wipe Quench	Wipe Sample	IPA	1	Dahlgren Decon™, EasyDECON® DF200, Decon PLUS™	No
	Wipe Sample	IPA	1		Yes
Rinsate Quench	Rinsate Sample	Toluene	1	Dahlgren Decon™, EasyDECON® DF200, Decon PLUS™	No
	Rinsate Sample	Toluene	1		Yes

Coupon and Wipe Extraction Efficiency Study Dosing Levels = 0.0004, 0.004, 0.04 and 0.2 milligram (mg)
Quench Extraction Efficiency Study Dosing Level = 0.02 mg
ABS: acrylonitrile butadiene styrene; HIPS: High impact polystyrene

2.2.1.1 Material Extraction Efficiency Study

The extraction efficiency of A-234 from each of the four material substrates was determined using isopropanol as the solvent and with three replicate samples for each material-contamination level combination. For each material (acrylonitrile butadiene styrene [ABS], silicone, Gorilla Glass®, and high impact polystyrene [HIPS]), four contamination densities (i.e., dosing levels) were used to achieve 0.02%, 0.2%, 2% and 10% of the target starting contamination amount of 2 g/m^2. The dosing levels (0.0004, 0.004, 0.04 and 0.2 g/m^2) were applied using dilute standards of A-234 in isopropanol (IPA). The study also included three spike controls at each contamination level to establish the baseline mass delivered, against which the extracted values would be compared.

The spike controls were prepared by delivering 2 µL of each dosing solution (A-234 in IPA) into 20-milliliter (mL) of IPA. Three replicate coupon samples (of each material type) were placed into 180-mL glass jars (Qorpak, P/N 239227, Clinton, PA) and contaminated with 2 µL of the appropriate dosing solution. The IPA was allowed to evaporate (typically < 1 min) and the coupon was extracted in 20 mL of IPA containing an internal standard (IS) (see Section 2.3.2). The extract jars were gently swirled and placed into a Branson Model 5510 ultrasonic bath (Emerson Electric Company, St. Louis, MO) and sonicated at 40 kilohertz (kHz) for 10 min. Extract aliquots were transferred to 2-mL liquid chromatography (LC) vials and analyzed for A-234 by Liquid Chromatography Tandem Mass Spectrometer/Spectrometry (LC-MS/MS).

2.2.1.2 Wipe Extraction Efficiency Study

A single wipe (Medi-Pak 7.5 centimeter [cm] x 7.5 cm Non-Woven Sponges 4-Ply, P/N Sterile-16-4234, Vitality Medical, Salt Lake City, UT) was placed into a 180-mL glass jar, wetted with 5

mL of IPA, contaminated with 2 µL of the appropriate dosing solution, allowed to dry for a maximum of 60 seconds, and extracted in 20 mL of solvent containing IS. The jars were gently swirled and placed into an ultrasonic bath for 10 min. Extract aliquots were transferred to 2-mL LC vials and analyzed for residual A-234 by LC-MS/MS.

2.2.1.3 Coupon and Wipe Quench Studies

Two quench studies were performed, one using stainless-steel coupons and the other using the solvent wipes. The experiments were designed to compare baseline extraction (i.e., no quench treatment) to treatment with a 3 molar (M) sodium thiosulfate (STS) quenching solution, an approach that had been used effectively in a previous CWA decontamination study [3].

Six stainless-steel (SS) coupons (2.5 cm x 4.0 cm) and six wipes were each placed into separate 180-mL glass jars (three replicates of each substrate for baseline and quench treatment). Decontaminant (1 mL) was delivered onto the surface of the coupon or wipe and allowed to reside for 60 min. The baseline samples received no further treatment at this time. Two 5-mL aliquots of quenching solution were added to the other samples. All samples (both baseline and quench) received 20 mL of IPA containing IS from a solvent dispenser. The solvent layers were each spiked with 2 µL of the A-234 dosing solution (0.02 milligram [mg] or 1% of the full challenge), gently swirled, and placed into an ultrasonic bath for 10 min. The jars were allowed to sit until the aqueous and solvent layers separated (approximately 30 min). From each jar, sample aliquots were drawn from the solvent layer and placed into 2-mL LC vials. The study also included three spike controls to establish the baseline mass delivered, against which the extracted values would be compared.

2.2.1.4 Rinsate Quench Study

The approach to this quench study was designed to compare baseline extraction (i.e., no quench treatment) to treatment with a 3M STS quenching solution. The rinsate quench study was developed using the following rationale: 1) the upper range of decontaminant volume on a test coupon surface was 100 µL/cm²; 2) rinsates from three replicate coupons were collected and combined into a single sample; 3) the expected total volume of rinsate was expected to be 12 mL, comprising 3 mL of decontaminant and 9 mL of water; and 4) the collected rinsates were divided into two equal samples. As a result, each rinsate test sample used for the study consisted of 1.5 mL of decontaminant and 4.5 mL of water (6 mL total). To replicate actual test conditions, the decontaminants used in the rinsate test samples were aged for 60 min prior to use.

The test samples receiving the quenchant were prepared as follows: 6 mL of rinsate test sample was placed into each 180-mL glass jar. Ten (10) mL of the quenchant (3M STS), then 20 mL of toluene containing IS were added to each jar. The solvent layer was spiked with 2 µL of the A-234 dosing solution to achieve 1% of the full challenge (0.02 mg). The jars were capped and shaken vigorously for 1 min. The layers were allowed to separate (generally around 30 min), and aliquots of the solvent layer were drawn and placed into 2-mL LC vials. The extracts were analyzed for residual A-234 using LC-MS/MS. All baseline samples were prepared exactly as described above, except that no quenchant was added. The study also included three spike controls to establish the baseline mass delivered, against which the extracted values would be compared.

2.2.1.5 A-234 Spiking Method Characterization

The accuracy of the contamination tool (described in Section 2.3.7) was determined gravimetrically using de-ionized (DI) water and a calibrated microbalance. Five 2-µL droplets of DI water were placed into a tared glass LC vial, and the weight was recorded. This was repeated five times, and the results were averaged. The results fell within ±10% of the expected value based upon the density of water (1.0 gram [g]/mL). Throughout the testing, A-234 spike controls were run with each test to provide continuous verification of the contamination tool's performance and accuracy.

2.2.1.6 Decontaminant Spray System Characterization

The decontaminant spray system (described in Section 2.3.9.4) was characterized and tuned to ensure it reproducibly applied the prescribed amount of each decontaminant (60 to 100 microliter per square centimeter [µL/cm^2]) over the coupon, panel, and SE item surfaces. The adjustable operating parameters included sprayer sweep speed, spray nozzle configuration, spray nozzle stand-off distance, and the programmed sweep pattern. Characterization was performed by placing eight tared glass petri dishes across the floor of the spray pan. The spray system was operated at a given set of parameters, and the mass of the decontaminant collected in each petri dish was weighed using a calibrated balance. The amount of decontaminant applied was calculated based upon the surface area of the petri dishes (149.5 cm^2), the density of the decontaminant, and the measured weight of the decontaminant. The spray system operating parameters were adjusted as necessary for each decontaminant to meet the requirements.

2.2.2 Decontamination Efficacy Evaluation

The decontamination efficacy evaluation was divided into five separate tasks (Task 2 through Task 6) to allow for real-time review of test data and test method adjustments for the successful decontamination of A-234.

- Task 2: These initial tests were conducted at a single set of conditions with the four selected SE materials (small coupons) and consisted of a simple process: contamination-weathering-decontamination-quenching-extraction-analysis.
- Task 3: This effort repeated the Task 2 experiments, but with the addition of a water rinse step after the 60-minute (min) decontaminant dwell time. The purpose was to compare efficacy results with and without the water rinse and to provide valuable information on potential post-decon rinsate contamination. Contaminated rinsate can have implications to post-event site remediation and waste disposal activities.
- Task 4: The results from Tasks 2 and 3 were used to implement modifications to the test methodologies to achieve decontamination efficacy improvements.
- Task 5: Decontamination efficacy tests were conducted to validate the methods optimized in Task 4 at a larger scale using panels of material. This task also incorporated field-relevant sampling techniques including wiping and blotting the material surfaces with wipes.

- Task 6: This task evaluated the final methodologies to demonstrate the performance of the decontaminants and procedures on actual SE items that may be used or are proxies for SE items used in an operational environment.

Test matrix tables for all tasks are presented in the following sections. The test trials for Tasks 2 through 5 included test samples, two positive controls, one negative control, one laboratory blank, and rinsate/overspray samples for each material type as shown in Figure 1.

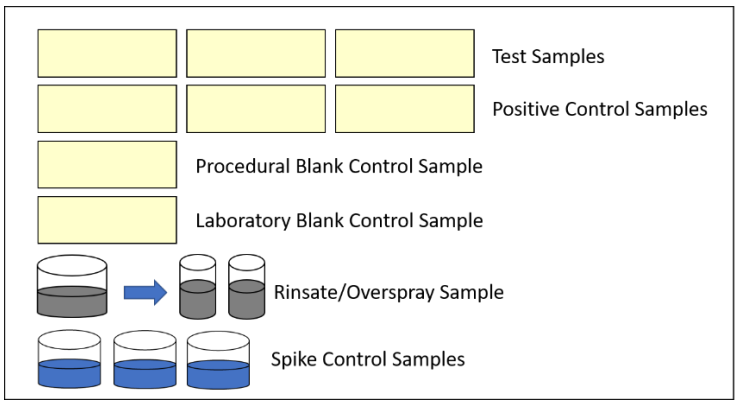

Figure 1. Test Trial Sample Types and Replicates

The sample types shown in Figure 1 are defined as:

- Test Sample: Received contamination followed by decontamination. Extracted (or wiped as appropriate) immediately at end of decontamination dwell time.
- Positive Control: Received contamination but not decontaminated. Extracted (or wiped as appropriate) immediately at the same time as the test samples.
- Procedural Blank: No contamination followed by decontamination. Extracted (or wipe sampled as appropriate) immediately at end of decontamination dwell time.
- Laboratory Blank: No contamination and no decontamination. Extracted (or wipe sampled as appropriate) immediately at the same time as the test samples.
- Overspray/Rinsate Sample: Combined into single sample from three replicates of the same material (for coupons and panels) or from five replicates (from full SE items). Resulting combined sample was divided into two samples with first getting immediate quench and extraction and the second being stored for 24 hours at room temperature, then quenched and analyzed. This allowed for an assessment of whether residual decontaminant in the rinsate resulted in further degradation of A-234.
- Spike Control: Contamination directly spiked into solvent for extraction and analysis.

The test samples, positive control samples, procedural blanks, and rinsate/overspray samples remained in the chemical fume hood throughout the experimental process. The laboratory blank samples were maintained outside of the fume hood until processing.

The number of Task 6 tests using full SE items and the number of sample replicates and positive controls was five. In addition to the above, all test trials included three spike controls.

2.2.2.1 *Task 2 – Initial Assessment of Decontamination Efficacy*

The test matrix for Task 2 is presented in Table 2. The material coupons were rectangular (2.5 cm x 4 cm) with a total surface area of 10 cm^2 as visible in Figure 2. Test coupons were contaminated as described in Section 2.3.8.1 and decontaminated as described in Section 2.3.9. The A-234 contact time and the decontaminant dwell time are shown in Table 2. The relatively short contact time prior to decontamination (60 min) may not align with a real response where decontamination may not occur for days. Extending the contact time was outside the scope of these assessments. Results for the short 60-min contact time are expected to be the same as for a prolonged contact time prior to decontamination due to the nonporous nature of the test materials that would leave the FGA on the surface with no or minimal permeation. Tests were conducted at ambient laboratory conditions, which were required to be between 18 and 24 degrees Celsius (°C) and 30 to 70% relative humidity (RH).

Table 2. Initial Assessment of Decontamination Efficacy (Task 2) Test Matrix

Test	Decontamination Technology	Material Types	A-234 Contact Time [min]	Decon Dwell Time [min]	Sample Replicates[a]	Decon Overspray Samples[a]
1[b]	Dahlgren Decon™	ABS Gorilla Glass® Silicone HIPS	60	60	3	2
2	EasyDECON® DF200					
3	Decon PLUS™					

[a] per material type

[b] test not performed due to problems with quenching and matrix interference (refer to Section 3.1.4)

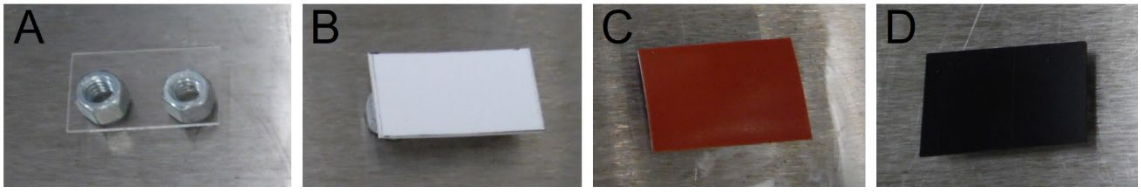

Figure 2. Test Material Coupons: (A) Glass, (B) HIPS, (C) Silicone, and (D) ABS

2.2.2.2 *Task 3 – Decontamination Efficacy with a Water Rinse*

The test matrix for Task 3 is presented in Table 3 below. The test matrix was identical to that of Task 2 except that a water rinse step was added after the decontaminant dwell time as described in Section 2.3.10). The Task 2 issues with the Dahlgren Decon™ (refer to Section 3.1.4) were eliminated with the water rinse step, allowing all three tests to be performed.

Table 3. Decontamination Efficacy with a Water Rinse (Task 3) Test Matrix

Test	Decontamination Technology	Material Types	A-234 Contact Time [min]	Decon Dwell Time [min]	Sample Replicates[a]	Decon Overspray Samples[a]
1	Dahlgren Decon™	ABS Gorilla Glass® Silicone HIPS	60	60	3	2
2	EasyDECON® DF200					
3	Decon PLUS™					
[a] per material type						

2.2.2.3 Task 4 – Modifications to Decontamination Efficacy Testing

The approach to Task 4 was decided based on the Task 2 and 3 results. Namely, one test would investigate the use of a water rinse only (i.e., no active decontaminant), the decontaminant dwell time would be extended from 60 to 120 min for the Decon PLUS™, and Dahlgren Decon™ would be used without the surfactant (Part A). The test matrix for Task 4 is presented in Table 4.

Table 4. Test Matrix for Modified Decontamination Efficacy Tests

Test	Decontamination Technology	Material Types	A-234 Contact Time [min]	Decon Dwell Time [min]	Sample Replicates[a]	Decon Overspray Samples[a]
1	Water Rinse Only – No oxidant applied	ABS Gorilla Glass® Silicone HIPS	60	60	3	2
2	Decon PLUS™			120		
3[b]	Dahlgren Decon™			60		
4[b]	Dahlgren Decon™ (retest)			60		
[a] per material type [b] minus surfactant Part A						

The purpose of Test 1 was to determine if the A-234 solubility in water was high enough such that it could be effectively removed from the surface of the SE materials and washed into the rinsate runoff. If proven effective, this approach could allow for a more aggressive approach to be used to treat the wastewater without the concern of damaging sensitive equipment. Test 2 was designed to determine if an extended dwell time for Decon PLUS™ could significantly increase its decontamination efficacy. This modification was not considered for EasyDECON® based on efficacy results from Tasks 2 and 3. The changes to the Dahlgren Decon™ approach (Tests 3 and 4, Task 4) were based upon its initial poor performance in Test 1 of Task 3, a phenomenon recognized by the distributor who indicated that the Dahlgren Decon™ has complications in degrading A-234. The distributor suggested that this might be attributed to a particular surfactant used in the decontaminant mixture interacting with the A-234 structure and recommended replacing the surfactant (Part A) with the same volume of DI water. This change was made for Tests 3 and 4. Test 3 was repeated (as Test 4) because excessive gas bubbles formed in the

sprayer feed line and caused the spray applicator to spit and sputter, resulting in a non-uniform application of the decontaminant.

2.2.2.4 Task 5 – Verification of Decontamination Efficacy Testing via Surface Sampling

Test panels (10.8 cm x 28 cm) of selected SE-related materials with a surface area of 302 cm^2 were used in this task. Following decontamination, residual A-234 was collected using surface wipe sampling techniques as described in Section 2.3.12, followed by normal solvent extraction and LC-MS/MS analysis. The target contamination density remained consistent with previous tasks and the number of 2-µL droplets of neat A-234 was increased (27 drops for a total of 54-µL) to achieve this objective. Further, it was decided that in Tests 5 through 8, a blotting step would be included to assist in the removal of excess liquid from the panel surface as described in Section 2.3.11. The blotting was conducted after the 60-min decontaminant dwell time and before the wipe sampling, to soak up the excess liquid that remained on the larger panels and provide a dryer surface for the wipe sampling process. Tests 7 and 8 had two sequential applications of decontaminant, each with a 60-min dwell time. The test matrix for Task 5 is presented in Table 5. Table 5 included the two decontaminants which resulted in highest observed degradation.

Table 5. Verification of Decon Efficacy via Surface Sampling (Task 5) Test Matrix

Test	Decontamination Technology (60-100 µL/cm²)	Material Type	A-234 Contact Time [min]	Decon Dwell Time [min]	Blotting Step Included	Replicates	Rinsate Samples
1	EasyDECON® DF200	HIPS	60	60	No	3	2
2	EasyDECON® DF200	Silicone	60	60	No	3	2
3	Decon PLUS™	HIPS	60	60	No	3	2
4	Decon PLUS™	Silicone	60	60	No	3	2
5	EasyDECON® DF200	HIPS	60	60	Yes	3	2
6	Decon PLUS™	HIPS	60	60	Yes	3	2
7	EasyDECON® DF200	HIPS	60	60 + 60	Yes	3	2
8	Decon PLUS™	HIPS	60	60 + 60	Yes	3	2

2.2.2.5 Task 6 – Decontamination Tests with Sensitive Equipment

Task 6 consisted of the experiments presented in Table 6. In this Task, iPhones (various models) and water-resistant calculators (representing soft material buttons on SE items such as handheld detectors) were selected as proxies for sensitive equipment. The water-resistant calculator, with its keyboard-like buttons, was selected as a proxy for devices that would be used in the response or consequence management phase and could become contaminated. As with Task 5, the number of A-234 droplets was adjusted to achieve a target contamination density of 2 g/m². Each test included five (5) replicate samples of two (2) SE items (iPhones and calculators) as well as the

required positive control (5) and blank (procedural and laboratory; 1 each) samples to evaluate decontamination efficacy. A combined rinsate/overspray sample was also collected for the test samples (5) and the procedural blank (1). For the calculator samples and positive controls, three (3) of the five (5) sample replicates were contaminated on the plastic casing and on top of the buttons, and the remaining two (2) replicate samples and positive controls were contaminated on the plastic casing and in the recessed areas of the buttons to demonstrate a worst-case scenario for decontamination should a piece of SE (by design or wear-and-tear) have recessed areas. The tests included two sequential applications of decontaminant, each with a 60-min dwell time. Post-decontamination residual A-234 determinations were made by employing surface blotting and wipe sampling techniques (i.e., solvent wipes) followed by extraction and analysis. An item function test was conducted for each SE item, both pre- and post-test, to determine potential and instant functional degradation caused by the decontaminants. The function test involved powering up the SE items to ensure proper function. The calculator buttons and functions, and iPhone touchscreens were tested for functionality. This test may indicate if a hand-held instrument can continue to be used, or in the case of iPhones or similar handheld devices, if the device is sufficiently operable to enable retrieval of its contents.

Table 6. Decontamination Tests with Sensitive Equipment (Task 6) Test Matrix

Test	Decontamination Technology (60-100 µL/cm²)	Material Type	A-234 Contact Time [min]	Decontaminant Dwell Times [min]	Blotting Step	Replicates	Rinsate Samples
1	Decon PLUS™	iPhone	60	60 + 60	Yes	5	2
	Decon PLUS™	Calculator				5	2
2	EasyDECON® DF200	iPhone	60	60 + 60	Yes	5	2
	EasyDECON® DF200	Calculator				5	2

2.3 Experimental Methods and Materials

Experimental methods and materials used to conduct the testing described in Section 2.2 are described in the subsections below.

2.3.1 Extraction Solvents

All solvents used for extractions and preparation of analytical standards were of pesticide grade purity (> 99% purity). Pesticide grade solvents are specially prepared and tested to avoid organic impurities at the parts per trillion (ppt) level. Manufacturer and lot information of each solvent was recorded as part of the test data sheets. Isopropanol (Fisher Chemical #A464-4, Fair Lawn, NJ) was used for the coupon and wipe extractions, and toluene (Fisher Chemical #T291-4, Fair Lawn, NJ) was used for the rinsate sample extractions.

2.3.2 Internal Standards (IS)

Diethyl (methylthiomethyl) phosphonate (DEMTMP) was used as an internal standard and was added to the extraction solvent at a concentration of 0.1 nanogram (ng)/µL. The IS concentration was chosen to fall within the mid-level range of the LC-MS/MS calibration curve, which

spanned 0.01 to 1.0 ng/µL (1-µL injections). Extraction solvent containing IS was prepared in 4-L batches prior to use for filling extraction jars. This is consistent with past EPA programs with traditional CWAs [3]. The DEMTMP (CAS No. 28460-01-7) was purchased from Sigma-Aldrich (Catalog No. 366668-25g) and had a listed purity of 96%. The stability of the IS was evaluated during the method demonstration/development and no degradation was observed during the experiments despite the potential for DEMTMP to be oxidized.

2.3.3 Solvent Dispensers, Electronic Pipets and Analytical Balances

A 2.5- to 25-mL Dispensette® S Bottletop Dispenser (BrandTech®, P/N 4600351) was used to fill extraction jars with 20-mL of solvent. The accuracy of the dispenser (±0.5 mL) was verified using a Class A 25-mL graduated cylinder before each test and recorded on the test data sheet. Delivery of quench solution and water rinses was performed using an Eppendorf Research Pro 100 to 5,000 µL electronic pipet (Eppendorf, Enfield, CT). The accuracy of the pipet was verified gravimetrically prior to each experiment. Five individual measurements were made by pipetting 5 mL of DI water into a tared 40-mL glass vial and recording the delivered mass. All five measurements were within ± 10% of the expected mass. All gravimetric measurements were conducted using a Model XS204 analytical balance (Mettler Toledo, Columbus, OH). The XS204 has a maximum capacity of 220 g with a readability and repeatability of 0.1 mg and linearity of ± 2 mg. Analytical balances are calibrated annually by Precision Scale & Balance (Lancaster, NY) and were checked prior to use with calibrated weights and were within ± 10% of the expected mass.

2.3.4 Sensitive Equipment Materials

Studies were conducted using the following types of SE-related materials: ABS, silicone, Corning® Gorilla Glass®, and HIPS. Test coupons measured 2.5 cm x 4 cm (10 cm²) and test panels measured 10.8 cm x 28 cm (302 cm²).

ABS (Curbell Plastics, #W01-00134-C, Orchard Park, NY) is a low-cost thermoplastic material with impact resistance, machinability, and thermoforming characteristics. ABS is used in a wide variety of products and applications, including electrical housings and containers. The coupons and panels were custom cut from a single 1.2 m x 2.4 m sheet.

The silicone (Rubber Cal, #02-W212-36RO-0062-5, Santa Ana, CA) material used for this project was a red/orange hard silicone sheet rubber with a 70 Shore A durometer rating. 70A rubbers are able to withstand physical indentation very well and are often used as a durable gasket and O-ring material for lab equipment and instrumentation and for commercial level chemical resistance. Silicone coupons and panels were cut from a single 1.0 m x 1.8 m sheet.

Gorilla Glass® (Valley Design Corp., #26024-1, Shirley, MA) is an alkali-aluminosilicate thin sheet glass that is well-suited to survive common glass failures. The composition allows a deeper layer of chemical strengthening that makes it more durable and scratch resistant. Gorilla Glass® is strengthened through a High Ion Exchange (HIE) process which creates a deep compression layer on the surface of the glass. Gorilla Glass® is used on electronic devices such as cellular phones, tablets, and touchscreen devices. Individual coupons and panels were custom cut from a single 1.2 m x 2.4 m sheet.

HIPS (United States Plastic Corp., #43334, Lima, OH) is a mechanically tough, low-cost plastic material that is easy to thermoform, fabricate, and machine. HIPS is used for medical devices

and equipment, displays and signs. HIPS coupons and panels were cut from a single 1.0 m x 1.8 m sheet.

Table 7. Sensitive Equipment-Related Materials

Material	Description	Supplier	Dimensions
ABS	General purpose ABS sheet (1.22 meter [m] x 2.44 m sheet), black, smooth surface	Curbell Plastics, Orchard Park, NY	Coupon: 2.5 cm x 4.0 cm Panel: 10.8 cm x 28 cm Thickness: 3 millimeter (mm)
Gorilla Glass®	Alkali-aluminosilicate thin sheet glass, HIE-strengthened	Valley Design, Shirley, MA	Coupon: 2.5 cm x 4.0 cm Panel: 10.8 cm x 28 cm Thickness: 1.1 mm
HIPS	High impact styrene sheet (1.83 m x 1.02 m sheet), opaque matte finish	United States Plastic Corp., Lima, OH	Coupon: 2.5 cm x 4.0 cm Panel: 10.8 cm x 28 cm Thickness: 1.5 mm
Silicone	100% Silicone sheet, red color, 70 Shore A, smooth finish	Rubber Cal, Santa Ana, CA	Coupon: 2.5 cm x 4.0 cm Panel: 10.8 cm x 28 cm Thickness: 1.59 mm

2.3.5 Description of SE Items

The iPhones and calculators were utilized as proxies for actual SE items that may be used by first responders during a Chemical, Biological, Radiological, and Nuclear (CBRN) emergency response. The iPhones represented common devices that first responders may use in the field to run instrumentation or gather data, and the calculators were selected to simulate equipment that has similar materials and buttons. The iPhones were in service prior to the experiments and showed normal wear-and-tear. Prior to the experiments, protective screens (if present) were removed and all iPhones were wiped with a clean cloth to remove any smudges and debris and charged to ensure proper power-up and function. Calculators were used straight from the box provided by the manufacturer and were powered up and tested for proper function before use.

Table 8. Sensitive Equipment Items

Material	Description	Supplier	Dimensions
iPhones	Various models: 6S, 7, 8, and XR	Apple Inc, Cupertino, CA	6S: 6.7 cm x 13.8 cm 7: 6.7 cm x 13.8 cm 8: 7.7 cm x 15.8 cm XR: 7.5 cm x 15 cm
Calculators	Victor® 99901 TuffCalc™ (white)	Victor Technology, LLC, Bolingbrook, IL	12 cm x 17 cm x 4.6 cm

2.3.6 Environmental Conditions

During testing, the laboratory was maintained within a range of 18°C to 24° C and 30% to 70% RH. Temperature and RH measurements were made and logged using a high-accuracy temperature and humidity probe (Lascar, Part No. EL-21CFR-2-LCD+). The instrument was calibrated at the factory and is traceable to the National Institute of Standards and Technology (NIST). The dynamic ranges are 0 to 100% with an accuracy of ± 2.25% for RH and -35 to +80 °C with an accuracy of ± 0.5 °C. Its sampling rate was set to one sample every ten min.

2.3.7 A-234 and A-234 Contamination Tool

Avarint synthesized all quantities of A-234 (ethyl N-[1-(diethylamino)ethylidene]-phosphoramidofluoridate, $C_8H_{18}FN_2O_2P$) used for this work. Synthesis was conducted in two batches, and each was analyzed for purity using GC-MS. The purity of A-234 was determined by both confirming the structure based upon the electron ionization (EI) mass fragmentation and by determining the percent area of the A-234 chromatographic peak versus any impurities present in the chromatogram. The lot number and purity were tracked and recorded for each test trial. The A-234 was stored under refrigeration at < 4° C in sealed glass vials until needed for testing. Purity checks were repeated when the A-234 was stored more than 30 days. The purity range of both A-234 lots throughout the testing is described in the table below.

Table 9. A-234 Purity Information

Chemical	Lot #	Purity [%]
A-234	CUB055-021921-Avarint	> 99
	CUB055-011422-Avarint	> 99

Contamination with A-234 was performed using a Model 1700 gas-tight syringe (Hamilton Company, Reno, NV) equipped with a 22-gauge blunt tip needle. The syringe was mounted into a Hamilton Model PB-600-1, fifty-step repeating dispenser. When used with a 100-µL syringe, 2-µL droplets of A-234 were delivered with each click of the dispenser. Together, these two items are referred to as the contamination tool.

2.3.8 Contamination

2.3.8.1 Small Coupon Contamination

Test coupons were placed into a 6.3-cm diameter glass petri dish (Figure 3) and elevated off the bottom using a 0.6-cm thick stainless-steel spacer to separate them from the runoff and overspray. The petri dish and coupon were placed onto the rack inside the sprayer pan and within the previously determined field of spray. The test coupons and positive control samples were contaminated with a single 2-µL drop of neat A-234 in the center of the coupon. At the same time, the spike control samples were generated by delivering the same quantity of A-234 directly into 20 mL of extraction solvent. Following contamination, the coupons were covered to protect from hood ventilation airflow and allowed to reside for the 60-min contact period.

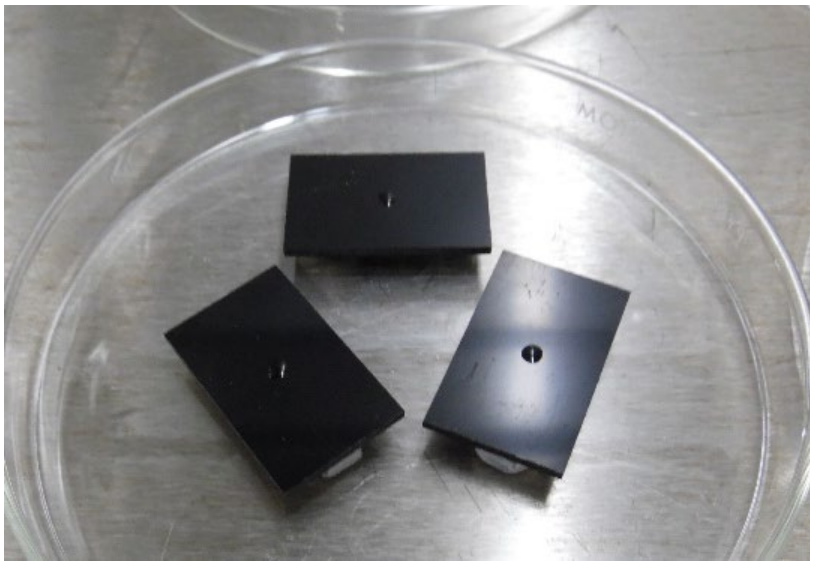

Figure 3. Example of Small Coupon Contamination (ABS material) with a single 2-µL drop of neat A-234

2.3.8.2 Panel Contamination

Test panels were placed into a 38-cm x 30-cm stainless-steel pan and were elevated off the bottom using 0.6 cm thick stainless-steel spacers (Figure 4). The pan and panels were set onto the rack inside the sprayer pan and within the previously determined field of spray. The test panels and positive control samples were contaminated with 27 2-µL droplets of A-234 evenly dispersed across the surface of the test panel in three rows of nine drops. The spike control samples were generated by delivering five 2-µL drops of A-234 directly into 20 mL of isopropanol. A lower number of droplets was used for the spike controls to reduce the quantity of A-234 consumed (and also eliminate the need to perform multiple dilution steps to be within range of the LC-MS/MS calibrations). Following contamination, the stainless-steel pan was covered to protect the samples from hood ventilation airflow and allowed to reside for the 60-min contact period.

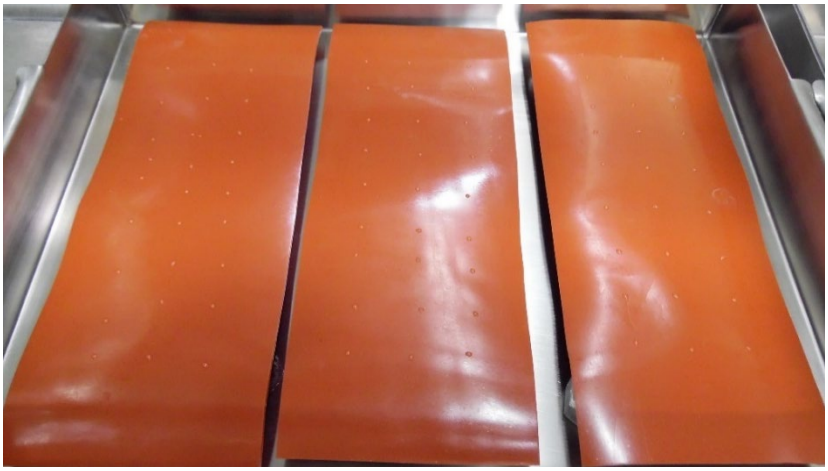

Figure 4. Example of Large Panel Contamination (Silicone material)

2.3.8.3 Sensitive Equipment Contamination

In the field, it is highly unlikely that any piece of equipment, when contaminated, would receive contamination on every surface (e.g., the bottom surface remains uncontaminated by the release). Therefore, the number of 2-µL drops and drop locations for each item type were placed across the surface evenly at locations of likely contamination (e.g., where buttons would be pressed).

The test items were placed into a 38-cm x 30-cm stainless-steel pan and elevated off the bottom using stainless-steel spacers. The pan and SE items were set onto the rack inside the sprayer pan and within the previously determined field of spray. The test items and positive control samples were contaminated using the contamination tool as described below:

- There were several models of iPhone used and due to the varying dimensions, prior to testing, it was decided the smallest models (6S and 7) would be used to determine the number of drops required to achieve the target contamination density of 2 g/m². The iPhones were contaminated on their glass surfaces with eight 2-µL drops. The outer edges with recessed areas at the top and bottom of the phones were avoided as shown in Figure 5.

- Two different contamination strategies were employed for the calculators. The calculators have buttons with recessed areas around them. It was decided that three calculators receive contamination on top of the buttons and two receive contamination in the recessed areas around the buttons. The calculators were contaminated with 18 2-µL droplets of the A-234. Three droplets were placed on the hard plastic material just above the keyboard and the remaining 15 droplets were placed either on the buttons (Pattern #1, Figure 5) or in the recessed areas (Pattern #2, Figure 5). Spike control samples were generated by delivering five 2-µL droplets of A-234 directly into 20 mL of isopropanol. Following contamination, the SE items were covered to protect them from hood ventilation airflow and were allowed to reside for the 60-min contact period.

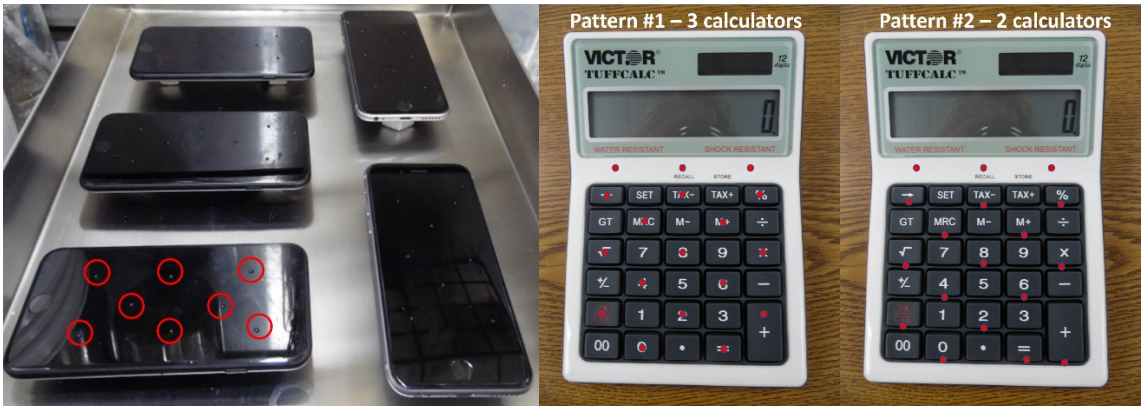

Figure 5. Example of iPhone (left) and Calculator (right) Contamination. Some deposited droplets are visible on the iPhone screens. The pattern is shown on one screen for reference. Red dots mark contamination locations for the calculators.

2.3.9 Application of Decontamination Technologies

The decontaminants evaluated are listed in Table 10 and are described in the following paragraphs. Decontaminants were procured directly from the manufacturer or supplier. The decontaminants were applied as liquids using the decontamination spray system (see Section 2.3.9.4).

Table 10. Tested Decontamination Technologies

Decontaminant	Manufacturer	Description
Dahlgren Decon™	First Line Technology	22-oz. kits
EasyDECON® DF200	Intelagard, Inc.	2-gal kits (PN 200-5312)
Decon PLUS™	ADS, Inc.	18- and 48-oz. kits

2.3.9.1 Dahlgren Decon™

Dahlgren Decon™ (DD-006-RTU, First Line Technology, Chantilly, VA) is a three-component decontaminant system including water and a surfactant package (Part A), sodium hydroxide (Part B1), and peracetyl borate (Part B2). The mixture generates peroxyacetic acid, a peroxy reactant, upon dissolution in water. The Part A solution is mixed with Parts B1 and B2. The manufacturer specifies that the unmixed components have a 10-year shelf life, and the pot life of a fully mixed and prepared solution is six hours.

The Dahlgren Decon™ was prepared in 22-oz. batches by emptying the Part A pouch into a large mixing container. Part B1 was carefully poured into Part A and stirred until the B1 particles were completely dissolved using the manufacturer-provided stirring rod. Part B2 was gradually added to the solution with continued stirring. The foaming reaction was allowed to settle before the solution was transferred into the sprayer reservoir. The decontaminant solution was used between 30 and 60 min after preparation.

2.3.9.2 EasyDECON® DF200

EasyDECON® DF200 (200-5312, Intelagard, Lafayette, CO) is a commercial variant of the Sandia National Laboratories decontamination foam DF200. EasyDECON® DF200 is a three-component technology containing water and water-soluble cationic surfactants (Part 1), hydrogen peroxide (Part 2) and diacetin (Part 3). Hydrogen peroxide (concentration of 8%) is the active ingredient, and diacetin (CAS No. 25395-31-7) is a catalyst that activates the hydrogen peroxide to form a peroxy reactant. The decontaminant can be applied as a liquid or a foam and was used as a liquid under this project. The manufacturer claims that the EasyDECON® DF200 solution will remain effective for 8 hours.

EasyDECON® DF200 was prepared in 2-gallon batches by emptying container Part 1 into a large, clean mixing container. Part 2 was then carefully poured into the mixing container and stirred. Part 3 of the EasyDECON® DF200 was added to the mixing container and gently stirred using the provided stir stick until all components were thoroughly blended. Then, the solution was transferred to the spraying reservoir to await spray application. The decontaminant solution was used between 30 and 60 min after preparation.

2.3.9.3 Decon PLUS™

The Decon PLUS™ (ADS, Inc., Virginia Beach, VA) kits include three pouches packaged in laminated foil packages. The components are stored as dry powders to be mixed at the point of use with available water sources. When the Part 1 (oxidant) component is dissolved in water, it releases hydrogen peroxide. The Part 2 (activator) component contains materials that react with the hydrogen peroxide to produce a peroxy reactant. The activator also contains a blend of surfactants and a buffer to control the pH. There is a Part B component that can be added for use against biological agents (it shifts a chemical equilibrium to boost the peroxyacetic acid concentration and adjusts the pH to enhance antimicrobial efficacy). Part B was not used in the testing. As used for this testing with A-234, the peroxyacetic acid will be in the 10,000 to 11,000 mg/L range, and the hydrogen peroxide concentration will be approximately 7,000 to 8,000 mg/L, with a pH between 9 and 10.

Decon PLUS™ was prepared by placing either 530 mL (to make 18 fluid ounces [fl. oz.]) or 1.4 liter (L) (to make 48 fl. oz.) of DI water into a mixing container. The larger 1.4L kits were used for later tests (Tasks 5 and 6) where multiple decon applications were conducted. The Part 1 component was added and stirred briefly. The Part 2 component was added to the solution and mixed thoroughly. The decontaminant solution was used between 30 and 60 min after preparation.

2.3.9.4 Decontaminant Spray System

The Decontaminant Spray System (DSS) was designed and built at Avarint to accommodate the selected liquid decontaminants and to provide a decontaminant application density of 60 to 100 $\mu L/cm^2$. A spray nozzle from a model HGSP01H 20V 2-gallon backpack sprayer (Hart Consumer Products, Inc., Anderson, SC) was attached to a metal track frame mounted above a 91-cm x 38-cm high-walled stainless-steel decontamination pan (see Figure 6). The sprayer head is powered using a 20V rechargeable battery and was fitted with long, flexible tubing to allow the backpack reservoir to remain outside of the fume hood during the testing. The DSS utilizes an X-Carve 1,000mm Computer Numerical Control (CNC) router platform (Inventables, Inc., Chicago, IL), and a programmable controller that directs the movement of the sprayer head across a defined area within the contamination pan. Both the movement pattern and speed of movement were programmable to achieve the desired application density of the decontaminants. The decontamination pan was designed with removable front panels to facilitate sample placement, contamination, sample processing and post-test cleanup. The floor area was large enough to accommodate all items prescribed for any given spray test.

Figure 6. Decontaminant Spray System Photos

Decontaminants were prepared as described in previous sections. Forty min following preparation, the pH was measured using Fisherbrand™ Hydrion™ Insta-Chek Display pH Paper (Fisher Scientific, Waltham, MA). The active ingredient levels were also determined using Hydrogen Peroxide and Peracetic Acid Test Kits (LaMotte, Chestertown, MD). Dahlgren Decon™ and Decon PLUS™ were tested using the peracetic acid kit, and EasyDECON® was tested using the hydrogen peroxide kit. Following characterization, the decontaminants were loaded into the sprayer reservoir, and the sprayer was primed by activating the pump for approximately 10 seconds (the initial decontaminant exiting the spray head during priming was collected and discarded). At this point, the DSS was ready for use.

Decontamination was performed by activating the decontaminant-specific program on the DSS. After the DSS completed its programmed spray pattern, the system was flushed with DI water to remove decontaminant and eliminate clogging within the sprayer head. In cases where multiple decontaminant sprays were conducted, the system was re-primed with decontaminant prior to use.

2.3.10 Water Rinse

The water rinse (when required) was performed by dispensing 3-mL of DI water (Evoqua Water Technologies, cascade system, Pittsburgh, PA) onto the sample surface using an Eppendorf Research Pro 100 to 5,000 µL electronic pipet.

2.3.11 Sample Blotting

Where required, a single dry Medi-Pak 7.5 cm x 7.5 cm Non-Woven Sponges 4-Ply, P/N Sterile-16-4234 (Vitality Medical, Salt Lake City, UT) wipe was dabbed across the surface of the test panel as illustrated in Figure 7 until the surface appeared dry and no more liquid appeared to absorb. The wipe was immediately placed into an extraction jar, quenched, and then extracted for analysis.

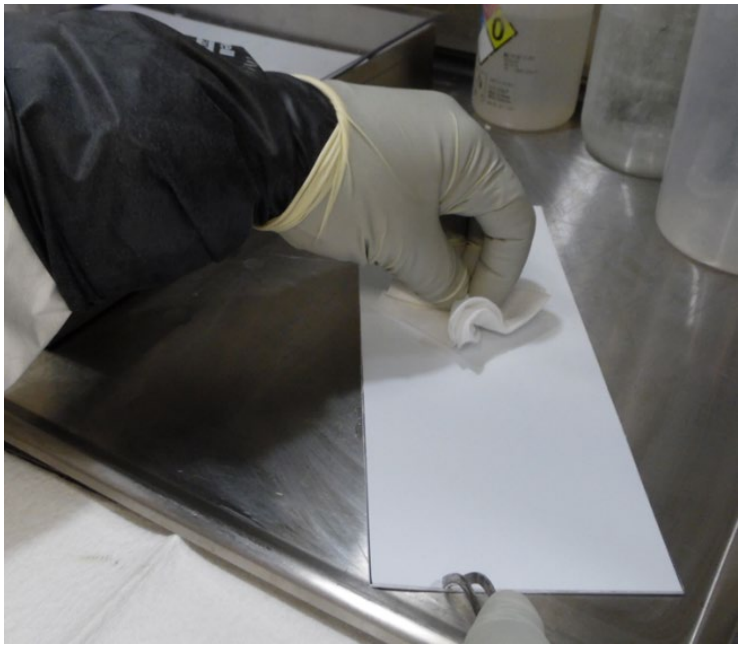

Figure 7. Blotting Step on HIPS Panel

2.3.12 Wipe Sampling

The number of required wipe passes was determined to ensure each sample type would receive thorough coverage. Table 11 presents these findings.

Table 11. Wipe Coverage Sampling Process

Test Sample Type	Horizontal Passes	Vertical Passes	Diagonal Passes
Panels	2	4	7
iPhones	2	3	4
Calculators	2	4	5

Prior to wiping, the test sample (panel or SE item) was tipped to allow excess decontaminant and/or rinse water to run off the surface and into the rinsate/overspray collection container. The wipe sampler described in the previous paragraph was wetted with 5 mL of isopropanol and used to perform overlapping wipe passes over the entire surface of the test item. Wipe passes were made (see Figure 8) horizontally, vertically, and diagonally across the surface of the test sample, and the wipe was folded between each change in direction to expose a clean area of the fabric. Passes were made with approximately 5-10% overlap with the previous pass. Any excess decontaminant remaining on the panel or SE item was absorbed into the wipe during the wiping action. Wipes were immediately placed into an extraction jar, quenched, and then extracted for LC-MS/MS analysis for residual A-234.

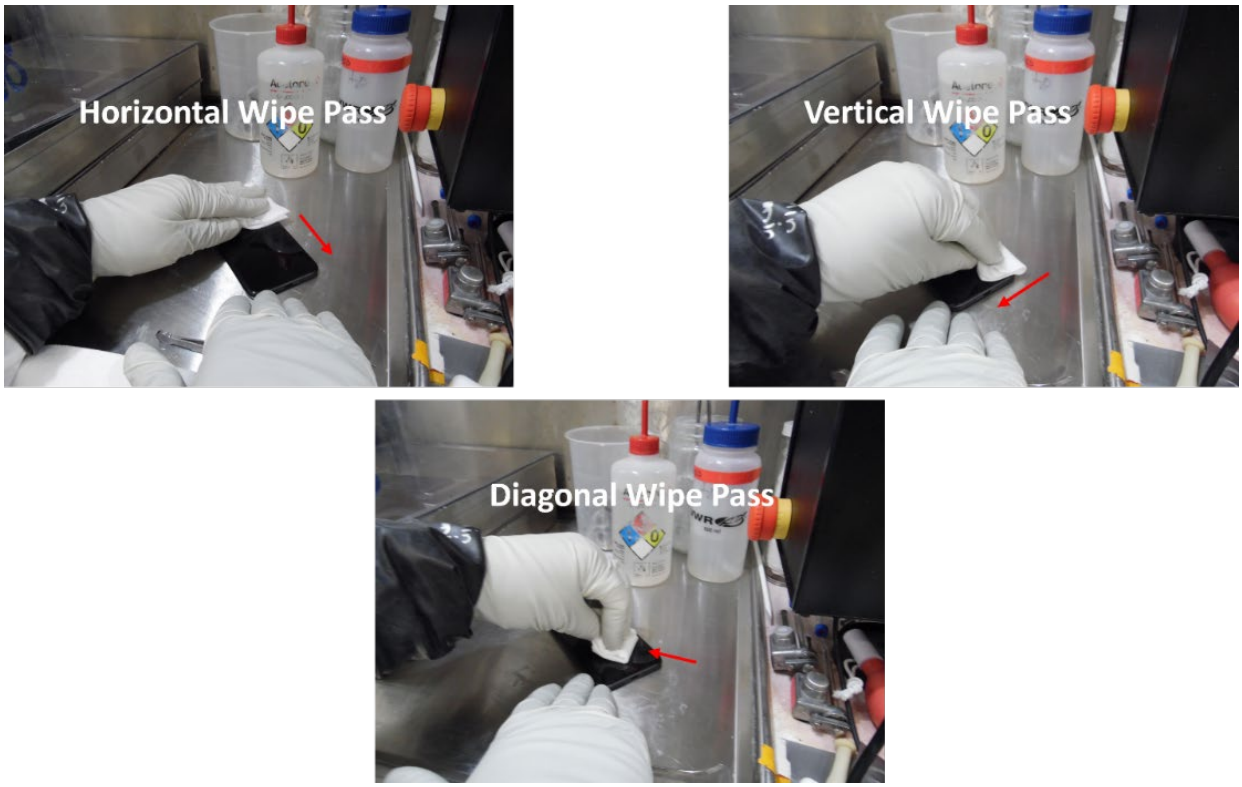

Figure 8. Example of Directional Wipe Passes (on iPhone)

2.3.13 Extraction of A-234 from Coupons and Wipes

Test coupons were tipped to allow excess decontaminant and/or rinse water to run off the surface and into the collection container. No other attempts were made to remove excess liquid decontaminant or water rinse remaining on the test sample. Samples were transferred and placed face-up into separate 180-mL glass jars, and 10 mL of the quenching solution was added to each followed by 20 mL of isopropanol containing the IS. The jars were capped, swirled for five seconds to mix, placed into a Branson Model 5510 ultrasonic bath (Emerson Electric Company, St. Louis, MO), and sonicated at 40 kHz for 10 min. An aliquot of the solvent layer from each jar was transferred into a 2-mL LC vial and analyzed by LC-MS/MS.

The wipe samples were extracted by placing the used wipe into a 180-mL glass jar and spreading the wipe evenly across the bottom of the jar. 10 mL of quenching solution was added to the jar followed by 20 mL of isopropanol containing IS. The jar was capped, swirled for five seconds to mix, and sonicated as described above. An aliquot of the extraction solvent from each jar was transferred into a 2-mL LC vial and analyzed by LC-MS/MS.

2.3.14 Rinsate/Overspray Sampling and Extraction of A-234

As discussed in previous sections, the decontaminant overspray and rinse water for all test material replicates were combined into a single sample. For the small coupon tests, the volume of overspray/rinsate was determined by transferring to a 25-mL Class A graduated cylinder (Fisher Scientific, Waltham, MA) using a glass Pasteur pipet. For the panel and SE item tests, the rinsate was poured into a 500-mL glass beaker and then poured into a Class A 250-mL graduated

cylinder (Fisher Scientific, Waltham, MA) to quantify the exact volume of the rinsate. Two 10-mL aliquots of the combined rinsate were placed into separate 180-mL glass jars and labeled. If the total volume of the overspray/rinsate was not greater than 20-mL, the sample volume was split evenly and placed into two separate jars. The first sample was quenched and extracted immediately for analysis. The second was capped and stored at room temperature for 24 hours, then quenched, extracted, and analyzed. This was performed by adding 10-mL of quench solution (3M STS) and 20-mL of toluene containing IS to the jar containing the sample. The jar was capped and shaken vigorously for one (1) min. The layers were allowed to separate, and then an aliquot of the solvent layer was transferred into a 2-mL LC vial and analyzed for A-234 by LC-MS/MS.

2.4 Analytical Methods

All analyses of sample and rinsate/overspray extracts were completed via LC-MS/MS. Methods were developed specifically for analysis of the A-234 analyte and IS used during testing.

2.4.1 Quantitative A-234 Analysis

Coupon and rinsate/overspray extracts were analyzed using LC-MS/MS to quantify the residual mass of A-234 present. A TSQ Endura™ (Thermo Fisher Scientific, Waltham, MA) Triple Quadrupole Mass Spectrometer with a heated electrospray ionization source (H-ESI II) coupled to a Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC UHPLC system was used for all sample analyses. Prior to use, the instrument was tuned and calibrated over a specified mass range (mass to charge ratio [m/z] = 69 to 1,522) in the positive ion mode by infusion of the Pierce™ Triple Quadrupole Calibration Solution (#88340). The MS-tune was conducted on a quarterly basis and repeated each time the instrument was modified, or repaired, or when maintenance was performed.

Multiple Reaction Monitoring (MRM) conditions were generated by infusion of a standard solution of A-234 at a flow rate similar to the flow rate used for the LC separation. The MS parameters (voltages, temperatures, gas flows, etc.) were adjusted to obtain optimal response and the current method parameters are shown in Table 12. Under this project, the LC conditions were adjusted to optimize peak resolution and shape for the matrices in use. DEMTMP was used as the IS for quantitation of A-234 and was added to the extraction solvent and calibration standards prior to LC-MS/MS analysis (nominal concentration in samples was 0.10 µg/mL). Detection was performed using positive ion electrospray ionization with MRM analysis using two ion transitions. The ratio of the ions monitored was tracked to ensure no interferences were present. The analyte areas of the primary (P) and secondary (S) ion transition ratios were monitored in all analyses and had to be within ± 50% of the average P/S ratio. A third transition ion was available in case of potential interferents with the P/S ion transition ratios, but otherwise was not used during analysis. Thermo Scientific™ Xcalibur software was used to control the instrumentation, the analysis of samples, and to acquire and process data.

Table 12. Wipe Coverage Sampling Process

Parameter	Condition		
Instrument	Thermo Scientific TSQ Endura™		
Ion Source	HESI-II Probe		
Spray Voltage (Volt [V])	3,500		
Vaporizer Temperature (°C)	317		
Ion Transfer Tube (°C)	333		
Sheath Gas Pressure, Nitrogen (Arb)	40		
Ion Sweep Gas Pressure (Arb)	1		
Auxiliary Gas Pressure, Argon (Arb)	12		
Capillary Temperature (°C)	333		
Collision Pressure (Arb)	1.5		
Analyte	**Ionization Mode**	**Precursor Ion (m/z)**	**Product Ion (m/z)**
A-234	Positive	225	197 (P)
	Positive	225	124.1 (S)
	Positive	225	74.3
DEMTMP (IS)	Positive	199	61 (S)
	Positive	199	143 (P)
UHPLC Conditions:			
Instrument:	Dionex Ultimate™ 3000 RS UHPLC System		
Column:	Accucore™ C18 (100 x 2.1 mm, 2.6 µm)		
Mobile phase A:	H_2O/ 0.1 % formic acid		
Mobile phase B:	Acetonitrile/0.1% Formic acid		
Gradient Profile	**Time, Min**	**%B**	**Flow Rate (ml/min)**
	0	5	0.3
	1.0	5	0.3
	3.0	95	0.3
	4.0	95	0.3
	4.5	5	0.3
	6.0	5	0.3
Injection volume (µL)	2.0		
Divert Valve to waste	0 to 1.5 min		
UHPLC: Ultra-High Performance Liquid Chromatography			

2.5 Calculations

2.5.1 Challenge Level Mass

The amount of A-234 applied to the test samples and controls was determined through the analysis of the spike control samples. The results of the three replicate spike control samples were used to provide a final value for the mass delivered for each spray test, calculated using the equation below:

$$M_D = (SC_E \times V_E)/10^6 \quad (1)$$

Where:

M_D = mass delivered in mg
SC_E = average concentration of spike control replicates in ng/mL
V_E = volume of extraction solvent in mL

2.5.2 Residual Agent Mass

The concentration of residual A-234 in solvent extracts was determined through the analysis of samples using LC-MS/MS. The results of the three replicates were used in the following equation to calculate the total mass of residual FGA:

$$RA_M = (RA_E \times V_E)/10^6 \quad (2)$$

Where:

RA_M = residual A-234 mass within extraction solvent in mg
RA_E = A-234 concentration within solvent extract volume in ng/mL
V_E = volume of extraction solvent in mL

2.5.3 Decontamination Efficacy

Decontamination efficacy was calculated using the equation below:

$$D = 1 - \frac{RA_M}{\bar{M}_{PC}} \times 100 \quad (3)$$

Where:

D = percent decontamination efficacy
RA_M = residual A-234 mass within extraction solvent in mg
M_{PC} = average mass recovered from positive controls in mg

Separate efficacy calculations were made for each material type and replicate. Decontamination efficacy results from replicate samples of each material-A-234 combination are presented individually and averaged (mean calculation).

2.6 Statistical Analyses

For each test condition, as defined by the decontamination technology/material type/decontamination period/sampling process, mean and relative standard deviation (RSD) of the A-234 mass recovery from each test sample were calculated. Select groups were then evaluated using one-way analysis of variance (ANOVA) *F*-tests to determine if the means of recovered A-234 mass for the data sets were significantly different from each other. Calculations were conducted using Microsoft Excel's Data Analysis add-on package and the ANOVA single factor analysis tool ($\alpha = 0.05$). The null hypothesis that the means were equal was rejected if the

F-test p-value was ≤ 0.05. Rejecting the null hypothesis indicates that the recovered A-234 mass from at least one of the data indicators is significantly different.

Additionally, Tukey Honest Significant Difference (HSD) tests were performed on data sets where more than two conditions were compared. The Tukey-HSD test compares the absolute difference of means between each data set to the critical range. The critical range is calculated by multiplying the Q-value (taken from the Studentized Range Q-table) by the square root of mean variance within groups divided by the number of observations in one group. If the absolute difference of means (AD) is greater than the critical range (CR) then there is a significant difference between the data sets. If AD < CR, no significant difference was found.

$$AD = |\bar{x}_1 - \bar{x}_2| \quad (4)$$

Where:

AD = Absolute difference of means
$\bar{x}_1$ = mean of first data set
$\bar{x}_2$ = mean of second data set

$$CR = Q \times \sqrt{\frac{MS}{n}} \quad (5)$$

Where:

CR = Critical Range
Q = Q-value taken from Studentized Range Q-table (based on degrees of freedom)
MS = mean variance within groups
n = number of observations in a single group

Results of statistical tests conducted on experimental data can be found in Attachment C of this report. There are some limitations of the statistical analyses used in this report that should be noted. Most tests conducted in this report have only three replicates, which is not a large enough sample size to guarantee the full validity and confidence of statistical analyses performed on the data. Using a small sample size can lead to ANOVA tests having both Type I and Type II error rates higher than the common standards for statistical significance. The tests in this report may have a greater than 5% chance to detect a significant difference between conditions where none exists and may have only a low likelihood to detect a significant difference between conditions where one does exist. As a result, the conclusions drawn from statistical tests in this report should be treated with some degree of caution.

RESULTS

3.1 Methods Demonstration

3.1.1 A-234 Spiking Method Characterization

The accuracy of the contamination tool was determined to be 98.8% of the target mass with ± 0.005 RSD. The tool accuracy was continuously verified throughout testing via spike control samples. All spike control samples fell within the required range of 70 to 130% of the target value as established as a data quality objective prior to the start of research.

3.1.2 Decontaminant Spray System Characterization

The DSS characterization results for the three decontaminants are summarized in Table 13.

Table 13. Spray Characterization for Decontaminant Technologies

Decontaminant	Average Volume/Area [µL/cm²]	STD	%RSD
Dahlgren Decon™	81	±1.8	2.2
EasyDECON® DF200	84	±1.2	1.5
Decon PLUS™	92	±2.0	2.2

3.1.3 Material and Sampling Wipe Extraction Efficiencies

The material coupon and wipe extraction efficiency results are summarized in Table 14.

Table 14. Material and Wipe Extraction Efficiency Results

A-234 Dose Level	Spike Control Mass	Gorilla Glass®		Silicone		HIPS		ABS		Wipe	
		Average Mass	Recovery	Average Mass	Recovery	Average Mass	Recovery	Average Mass	Recovery	Average Mass	Recovery
g/m²	mg	mg	%	mg	%	mg	%	mg	%	mg	%
0.2	0.16	0.15	96 (0.33)	0.16	102 (3.3)	0.16	102 (1.9)	0.14	93 (0.65)	0.15	98 (2.8)
0.04	0.035	0.033	95 (1.7)	0.033	99 (7.6)	0.037	106 (4.5)	0.033	95 (3.8)	0.036	99 (2.0)
0.004	0.0034	0.0034	98 (2.0)	0.0033	96 (2.7)	0.0033	95 (4.5)	0.0032	93 (6.8)	0.0035	101 (2.3)
0.0004	0.00034	0.00032	95 (7.3)	0.00035	102 (6.5)	0.00035	102 (3.67)	0.00034	101 (4.3)	0.00026	78 (5.1)
%RSD for recovery presented in parentheses											

3.1.4 Coupon and Wipe Quench Method Development Test Results

The baseline data set was generated using isopropanol extraction solvent only (i.e., no quench solution, Table 15). The results showed that no quantifiable A-234 was recovered for any of the decontaminants, indicating that an active chemical neutralization of the decontaminants would be necessary. The results of the 3M STS quench method are summarized in Table 16.

Table 15. Baseline (No Quench) Solvent Extraction Test, Average Mass Recovery

Sample Description		Dahlgren Decon™		EasyDECON® DF200		Decon PLUS™	
		Mass (mg)	Recovery	Mass (mg)	Recovery	Mass (mg)	Recovery
Spike Controls	Avg	0.021	110%	0.020	99%	0.021	109%
	%RSD	3.1	-	4.6	-	6.3	-
Coupons (stainless steel)	Avg	ND	ND	ND	ND	ND	ND
	%RSD	-	-	-	-	-	-
Wipes	Avg	ND	ND	ND	ND	ND	ND
	%RSD	-	-	-	-	-	-
ND = Non detect for A-234							

Table 16. 3M STS Quench Method Demonstration Test, Average Mass Recovery

Sample Description		Dahlgren Decon™		EasyDECON® DF200		Decon PLUS™	
		Mass (mg)	Recovery	Mass (mg)	Recovery	Mass (mg)	Recovery
Spike Controls	Avg	0.021	108%	0.020	99%	0.022	109%
	%RSD	3.1	-	4.6	-	6.3	-
Coupons (stainless steel)	Avg	0.0046	22%	0.021	106%	0.022	101%
	%RSD	30	-	5.5	-	14	-
Wipes	Avg	0.0029	14%	0.020	103%	0.023	107%
	%RSD	35	-	5.9	-	3.9	-

The 3M STS solution was effective in quenching the EasyDECON® DF200 and Decon PLUS™ decontaminants, but not the Dahlgren Decon™, where the recoveries were 22% for the coupons and 14% for the wipes. At the time these studies were conducted, it was hypothesized that the low recoveries may be a result of inadequate quenching of the high peroxyacetic acid levels or potential analytical interferences from the matrix with the LC-MS/MS. It was also noted that after the analytical runs were performed with Dahlgren Decon™ samples, instrument variability was observed with test samples and calibration standards. The LC-MS/MS instrument required additional maintenance such as cleaning the ion transfer tube, RF lens, and replacement of the needle insert before additional analytical runs could be performed. It was later determined that inadequate quenching of the Dahlgren Decon™ may be attributable to its surfactant component (refer to Section 2.2.2.3). As such, alternate quenching solutions were investigated for the Dahlgren Decon™:

- 3M and 6M Tetrahydrothiophene (THT)
- 1M Ascorbic Acid
- 3M STS quench following a water rinse step as part of the decontamination procedure (to reduce the amount of decon on the sample prior to quenching)

The results of the alternate quench study are summarized in Table 17.

Table 17. Alternate Quench Method Demonstration for Dahlgren Decon

Sample Description		3M THT		6M THT		1M Ascorbic Acid		3M STS w/rinse	
		Mass (mg)	Recovery	Mass (mg)	Recovery	Mass (mg)	Recovery	Mass (mg)	Recovery
Spike Controls	Avg	0.021	103%						
	%RSD	3.9	-						
Coupons (stainless steel)	Avg	0.0011	5.3%	0.00094	4.6%	0.0013	6.5%	0.018	89%
	%RSD	19	-	29	-	9.5		28	-
Wipes	Avg	0.0013	6.5%	0.0010	5.2%	0.0018	9.0%	0.016	77%
	%RSD	1.3		0.84		44		8.5	

The 3M STS solution with a water rinse demonstrated an 89% and 77% average recovery from the coupon and wipe, respectively. These are substantially better quenching results than 3M STS solution alone (22% and 14% for coupon and wipe, respectively; Table 16). As such, it was selected for use with the Dahlgren Decon™ decontamination efficacy evaluations that included a water rinse of the surface.

3.1.5 Rinsate Extraction Efficiencies

The results of the rinsate quench study are summarized in Table 18.

Table 18. Rinsate Quench Method Demonstration Test, Average Mass Recovery

Sample Description		Dahlgren Decon™		EasyDECON® DF200		Decon PLUS™	
		Mass (mg)	Recovery	Mass (mg)	Recovery	Mass (mg)	Recovery
Spike Controls	Avg	0.024	119%	0.021	104%	0.022	110%
	%RSD	5.6		3.3	-	7.4	-
Rinsate	Avg	0.021	90%	0.021	102%	0.023	108%
	%RSD	4.8	-	12	-	3.2	-

The A-234 mass recoveries from the rinsate quench studies for all three decontaminant technologies ranged from 90% to 108% recovery and were within acceptable 70% to 130% expected mass ranges. The use of toluene solvent led to a separation of phases with the A-234 in the solvent phase while the active ingredients of the residual decontaminant remained in the aqueous phase. The toluene solvent could not be used for the coupon extractions due to incompatibilities with the SE materials being tested.

3.2 Decontamination Efficacy Evaluations

3.2.1 Task 2 – Initial Assessment of Decontamination Efficacy Tests

These tests evaluated the decontamination efficacy of EasyDECON® DF200 and Decon PLUS™ on small coupons. The spike control recoveries were 95% and 99% for the EasyDECON® DF200 and Decon PLUS™ tests respectively, demonstrating that the contamination tool was performing

within the established requirement. No A-234 was detected in any laboratory blank samples or in any of the rinsate procedural blanks. There was one low-level detection in the HIPS procedural blank sample in the EasyDECON® DF200 test; however, the detected amount was less than 0.01% of the corresponding positive control value for the HIPS material. The decontamination efficacy results are summarized in Figure 9, and the detailed data are presented in Table 19.

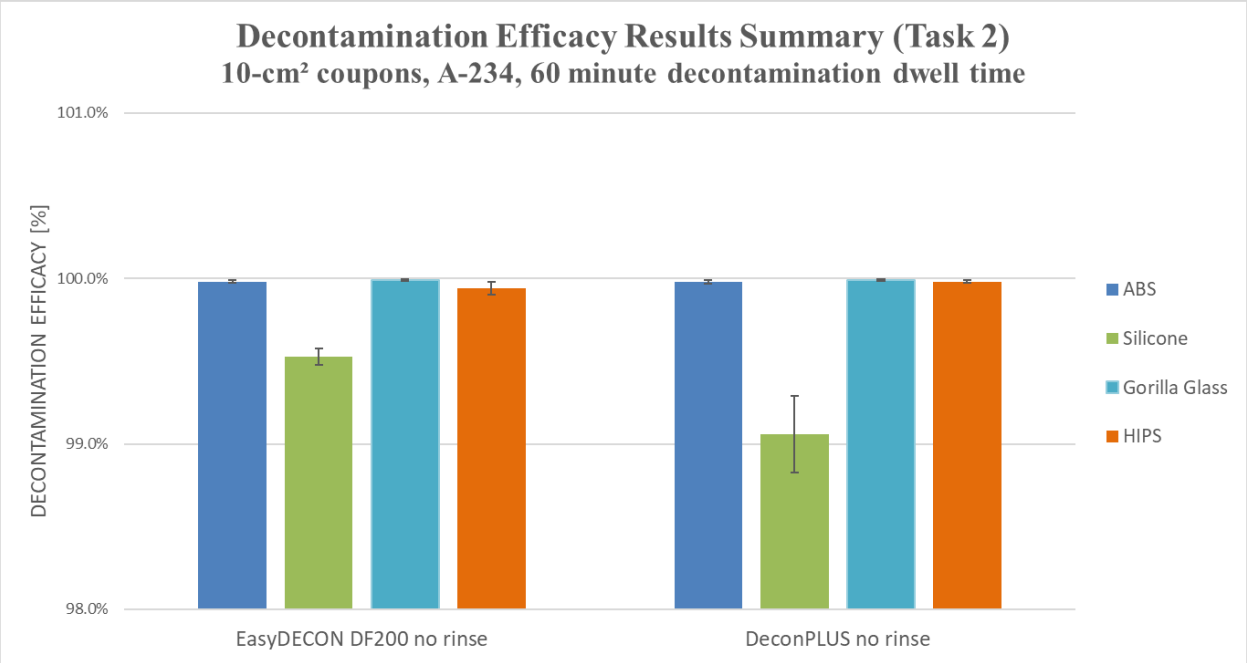

Figure 9. Decontamination Efficacy Results for Small Coupon Tests (Task 2)

Table 19. Decontamination Efficacy Results for Coupon Tests (Task 2)

Decon	Material	Sample Type	Mean [mg]	STDEV [mg]	RSD [%]	Efficacy [%]
EasyDECON DF200	ABS	Positive Controls	2.2	0.037	1.7	99.98 ± 0.01
		Test Coupons	0.00040	0.00015	39	
	Silicone	Positive Controls	2.2	0.070	3.2	99.53 ± 0.05
		Test Coupons	0.010	0.0010	10	
	Gorilla Glass	Positive Controls	2.2	0.014	0.64	99.99 ± 0.01
		Test Coupons	0.00023	0.00012	51	
	HIPS	Positive Controls	2.2	0.067	3.1	99.94 ± 0.04
		Test Coupons	0.0013	0.00082	65	
Decon PLUS™	ABS	Positive Controls	2.2	0.030	1.4	99.98 ± 0.01
		Test Coupons	0.00044	0.00027	61	
	Silicone	Positive Controls	2.1	0.12	5.5	99.06 ± 0.23
		Test Coupons	0.020	0.0048	24	
	Gorilla Glass	Positive Controls	2.0	0.13	6.3	> 99.99 ± 0.01
		Test Coupons	< 0.00018	0.00011	63	
	HIPS	Positive Controls	2.2	0.19	8.9	99.98 ± 0.01
		Test Coupons	0.00039	0.00020	51	

Table 20 shows the residual mass of A-234 in the rinsate sample for each material type as the average mass recovered for the samples that were quenched and extracted immediately post-decontamination and those that were quenched and extracted 24-hours later. Results are summarized in Figure 10.

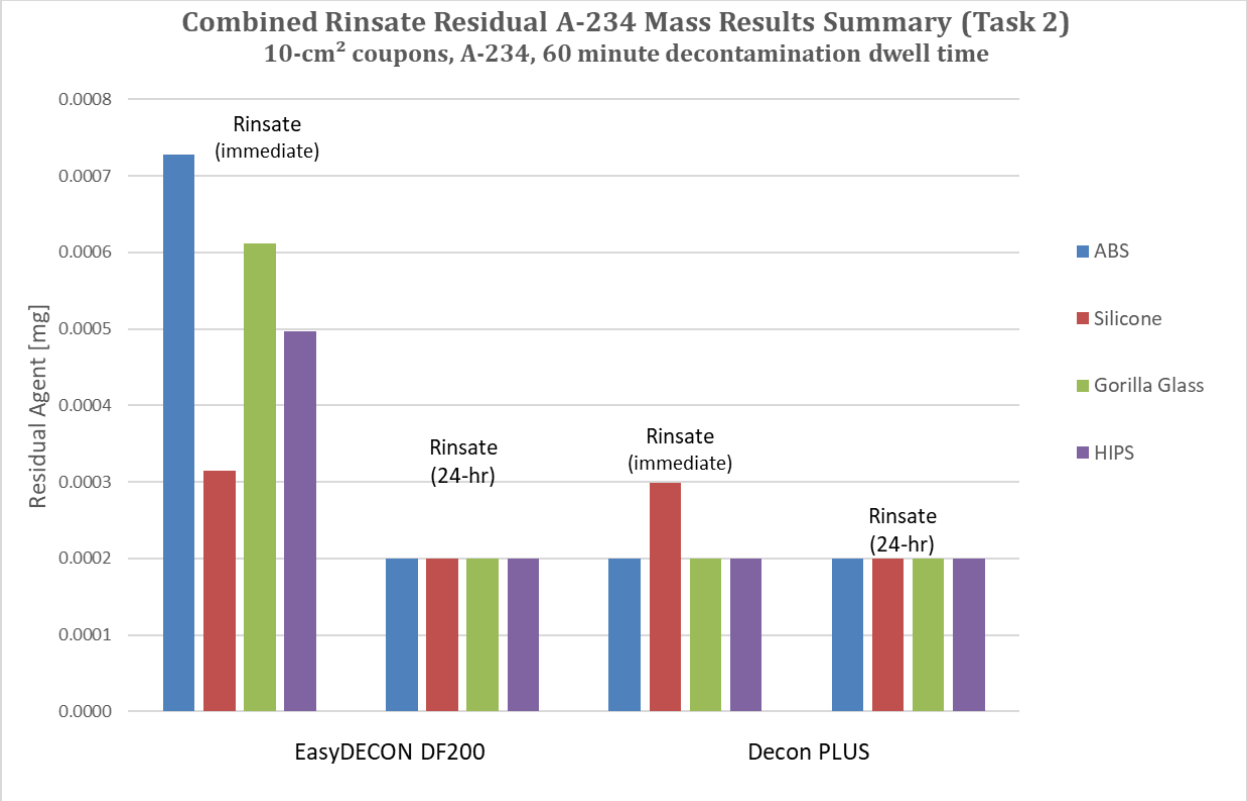

Figure 10. Residual A-234 in Decontaminant Overspray Samples (Task 2)

Table 20. Residual A-234 in Rinsate Samples for Coupon Tests (Task 2)

Decon	Material	Rinsate (immediate) [mg]	Rinsate (24-hr) [mg]
EasyDECON DF200	ABS	0.00073	< 0.00020
	Silicone	0.00031	< 0.00020
	Gorilla Glass	0.00061	< 0.00020
	HIPS	0.00050	< 0.00020
Decon PLUS™	ABS	< 0.00020	< 0.00020
	Silicone	0.00030	< 0.00020
	Gorilla Glass	< 0.00020	< 0.00020
	HIPS	< 0.00020	< 0.00020

The EasyDECON immediate rinsate samples, for all four materials, resulted in very low positive detections of A-234, but were <MDL for the 24-hour samples. This would imply that when used in the field and depending on the initial amount of A-234 present, there would initially be some

residual A-234 remaining in the collected rinsate/runoff; however, that amount decreases with time provided residual oxidant is not quenched.

3.2.2 Task 3 – Decontamination Efficacy with Water Rinse Tests

Task 3 repeated the Task 2 coupon tests with the addition of a water rinse and consisted of four tests:

- Test 1: EasyDECON® DF200
- Test 2: Decon PLUS™
- Test 3: Dahlgren Decon™
- Test 4: Dahlgren Decon™ (retest)

The second Dahlgren Decon™ test was performed because significant amounts of residual A-234 were found in the rinsate samples (see discussion below). The Dahlgren Decon™ used during tests 3 and 4 was prepared according to the manufacturer's instructions (i.e., contained Part A surfactant). The spike control results of 94%, 98%, 93%, and 105% for tests 1 through 4, respectively, demonstrated that the contamination tool was performing within the established requirements. No A-234 was detected in any laboratory and procedural blank samples nor any of the rinsate procedural blank samples. The decontamination efficacy results are summarized in Figure 11, and the detailed data are presented in Table 21. Efficacies were calculated by comparing the residual A-234 mass on the test coupons against the mass from the corresponding positive controls.

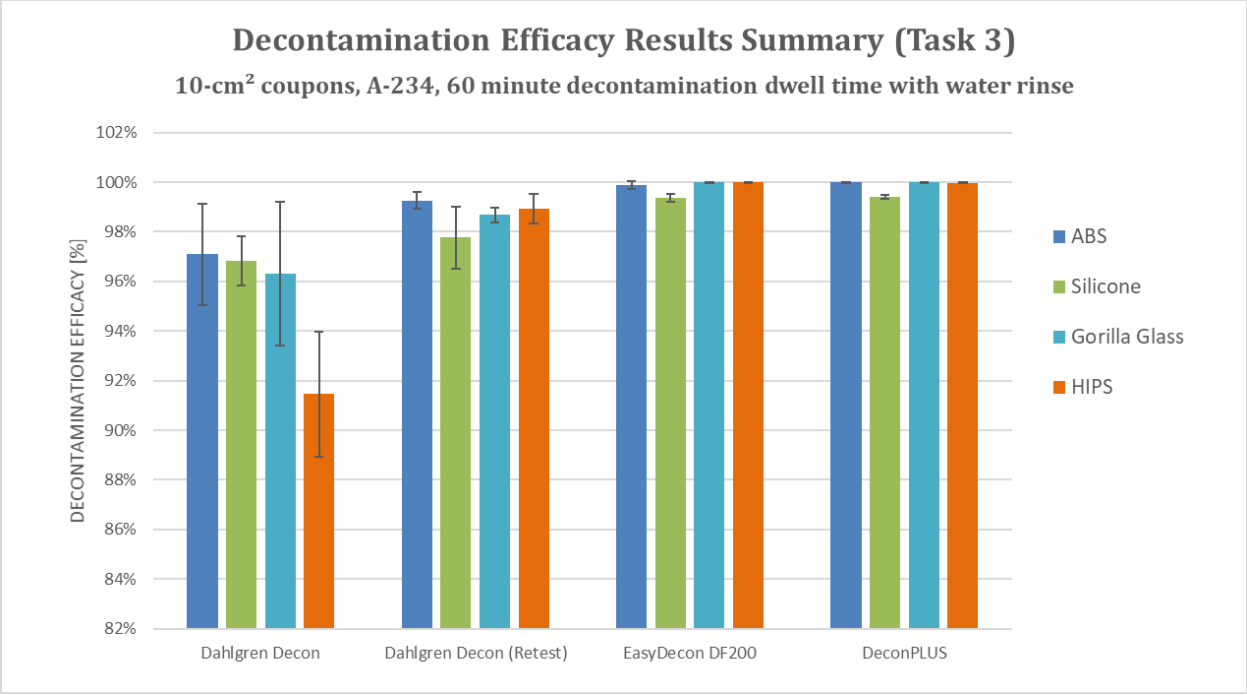

Figure 11. Decontamination Efficacy for Small Coupon Tests with Water Rinse (Task 3)

Table 21. Decontamination Efficacy for Coupon Tests with Water Rinse (Task 3)

Decon	Material	Sample Type	Mean [mg]	STDEV [mg]	RSD [%]	Efficacy [%]
Dahlgren Decon	ABS	Positive Controls	2.1	0.051	2.4	97.09 ± 2.0
		Test Coupons	0.060	0.042	70	
	Silicone	Positive Controls	2.2	0.14	6.5	96.82 ± 1.0
		Test Coupons	0.069	0.021	31	
	Gorilla Glass	Positive Controls	2.1	0.20	9.2	96.30 ± 2.9
		Test Coupons	0.079	0.061	77	
	HIPS	Positive Controls	2.1	0.12	5.9	91.46 ± 2.5
		Test Coupons	0.18	0.051	29	
Dahlgren Decon (Retest)	ABS	Positive Controls	2.3	0.051	2.3	99.26 ± 0.33
		Test Coupons	0.017	0.0075	45	
	Silicone	Positive Controls	2.4	0.088	3.7	97.77 ± 1.3
		Test Coupons	0.053	0.030	56	
	Gorilla Glass	Positive Controls	2.3	0.089	3.8	98.67 ± 0.30
		Test Coupons	0.031	0.0070	23	
	HIPS	Positive Controls	2.3	0.052	2.3	98.91 ± 0.59
		Test Coupons	0.025	0.014	54	
EasyDECON DF200	ABS	Positive Controls	2.0	0.075	3.7	> 99.88 ± 0.17
		Test Coupons	< 0.0025	0.0034	136	
	Silicone	Positive Controls	2.2	0.28	12	99.36 ± 0.16
		Test Coupons	0.014	0.0030	21	
	Gorilla Glass	Positive Controls	2.0	0.098	4.8	> 99.99 ± 0.001
		Test Coupons	< 0.00011	0.000020	17	
	HIPS	Positive Controls	2.1	0.064	3.1	> 99.99 ± 0.002
		Test Coupons	< 0.00016	0.000042	27	
Decon PLUS™	ABS	Positive Controls	2.3	0.10	4.3	> 99.99 ± 0.002
		Test Coupons	< 0.00014	0.000049	36	
	Silicone	Positive Controls	2.4	0.075	3.1	99.42 ± 0.079
		Test Coupons	0.014	0.0019	13	
	Gorilla Glass	Positive Controls	2.2	0.11	5.3	> 99.99 ± 0.002
		Test Coupons	< 0.00015	0.000039	26	
	HIPS	Positive Controls	2.2	0.13	5.6	> 99.97 ± 0.03
		Test Coupons	< 0.00075	0.00062	83	

The measured decontamination efficacy for the first Dahlgren Decon™ test was 91.5 to 97.1%, but analysis of the rinsate samples test showed an A-234 residual mass between 88.2 and 101% of the positive controls (mean mass of positive control x 3 replicates). The experiment and its details were reviewed and discussed by the test team and no deviations from the test procedures

were noted that could have caused the observed results. The test was repeated to determine if the same results would be observed.

Overall, the measured decontamination efficacies for the Dahlgren Decon™ ranged from 91.5% to 99.3% for the four material types. While decontamination efficacies in this range are considered relatively high, analysis of the immediate rinsate samples revealed that between 63% and 101% of the initial A-234 challenge (based on positive controls), from the three replicate samples, remained in the rinsate. In addition, no appreciable degradation of A-234 in the rinsate occurred over 24 hr. The potential mechanism for this is discussed in Task 4 below. Regardless, these results suggest that although the Dahlgren Decon™ may be effective at removing A-234 from SE surfaces by physical removal, A-234 may present challenges to full-scale operational remediation efforts because of potential hazards associated with significant A-234 remaining in the decontamination rinsates.

The rinsate analysis results from all four tests are presented in Figure 12 and the detailed data are provided in Table 22.

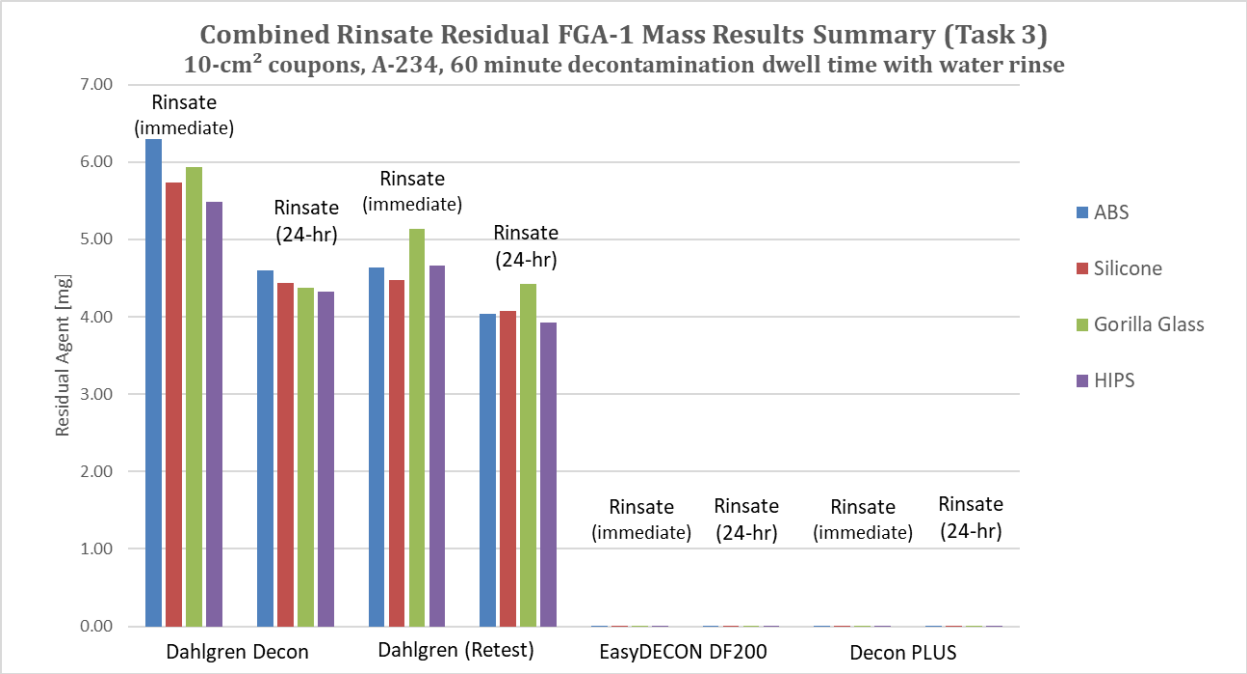

Figure 12. Residual A-234 in Rinsate Samples for Tests with Water Rinse (Task 3)

Table 22. Residual A-234 in Rinsate Samples for Coupon Tests with Water Rinse (Task 3)

Decon	Material	Rinsate (immediate) [mg]	Rinsate (24-hr) [mg]
Dahlgren Decon	ABS	6.3	4.6
	Silicone	5.7	4.4
	Gorilla Glass	5.9	4.4
	HIPS	5.5	4.3
Dahlgren Decon (Retest)	ABS	4.6	4.1
	Silicone	4.5	4.1
	Gorilla Glass	5.1	4.4
	HIPS	4.7	3.9
EasyDECON DF200	ABS	0.0022	< 0.00020
	Silicone	0.00040	< 0.00020
	Gorilla Glass	0.00020	< 0.00020
	HIPS	0.00024	< 0.00020
Decon PLUS™	ABS	0.00052	< 0.00020
	Silicone	0.00060	< 0.00020
	Gorilla Glass	0.00026	< 0.00020
	HIPS	0.0010	< 0.00020

3.2.3 Task 4 – Modifications to Decontamination Efficacy Tests

Task 4 consisted of four tests with procedural modifications to potentially increase decontamination efficacy. The tests consisted of:

- Test 1: Water Rinse Only – No oxidant applied
- Test 2: Decon PLUS™, 120-min decontaminant dwell
- Test 3: Dahlgren Decon™, minus surfactant (Part A)
- Test 4: Dahlgren Decon™ (repeat of test #3)

The spike control results of 97%, 106%, 94% and 90% for tests 1 through 4, respectively, demonstrated that the contamination tool was performing within the established requirements. There was a detection for A-234 in both the ABS procedural blank coupon and rinsate sample in both modified Dahlgren Decon™ tests (Test #3 and Test #4, retest). These positive detections were most likely caused by the "spitting" decontaminant spray application (discussed below). Detections in the ABS procedural and rinsate blanks were less than 1% of the associated positive controls for the ABS material. The decontamination efficacy results are summarized in Figure 13 and the detailed data are presented in Table 23. Efficacies were calculated by comparing the residual A-234 mass on the test coupons against the mass from the corresponding positive controls.

The spray application of the modified Dahlgren Decon™ had excessive gas bubble formation in the sprayer line, which caused the decontaminant spray to "spit" as it was being applied and resulted in uneven coverage across the spraying field. The spray system was evaluated post-test to determine if a malfunction of the spray system was the cause of the gas bubble formation during the Dahlgren Decon™ spray application. Post-test, the system was operated with DI water and the sprayer worked as expected, with no gas bubble formation or "spitting". The sprayer was also operated with the same Dahlgren Decon™ solution that was used during the test except the decontaminant solution was 24-hours old. The sprayer again operated as expected, with no gas bubble formation or "spitting" when using the aged Dahlgren Decon™ solution. Based on these system checks, it appears the gas bubble formation may be unique to the freshly prepared active Dahlgren Decon™ minus the surfactant, or that the surfactant prevents spitting associated with gas bubble formation. To verify this, the modified Dahlgren Decon™ test was re-run (Test #4). The same issue with gas bubble formation and the "spitting" spray occurred in the repeat test (Test #4). Based on the results, the DSS spray apparatus used for this testing was not able to provide a smooth and evenly distributed decontamination spray with the Dahlgren Decon™ when the surfactant is replaced with water. The uneven decontaminant spray application could negatively impact the decontamination efficacy results for the Dahlgren Decon™.

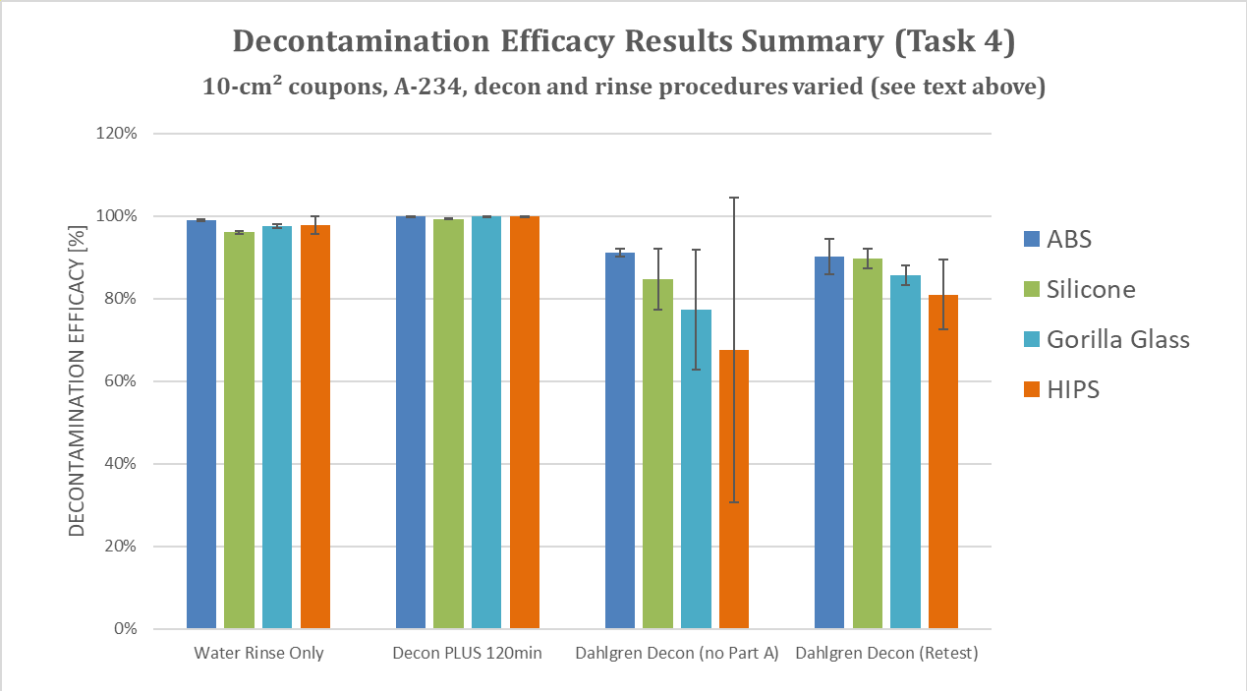

Figure 13. Decontamination Efficacy for Coupon Tests with Water Rinse (Task 4)

Table 23. Decontamination Efficacy for Coupon Tests with Water Rinse (Task 4)

Decon	Material	Sample Type	Mean [mg]	STDEV [mg]	RSD [%]	Efficacy [%]
Water Rinse Only – No Oxidant Applied	ABS	Positive Controls	2.1	0.11	5.3	98.92 ± 0.31
		Test Coupons	0.023	0.0064	28	
	Silicone	Positive Controls	2.0	0.10	5.0	96.00 ± 0.42
		Test Coupons	0.081	0.0075	9.2	
	Gorilla Glass	Positive Controls	2.1	0.036	1.8	97.50 ± 0.58
		Test Coupons	0.051	0.012	23	
	HIPS	Positive Controls	2.0	0.11	5.8	97.76 ± 2.1
		Test Coupons	0.045	0.042	95	
Decon PLUS™ – Age Time 120 min	ABS	Positive Controls	2.1	0.011	0.54	99.96 ± 0.03
		Test Coupons	0.00082	0.00072	88	
	Silicone	Positive Controls	2.2	0.069	3.1	99.20 ± 0.13
		Test Coupons	0.018	0.0029	16	
	Gorilla Glass	Positive Controls	2.2	0.047	2.2	99.95 ± 0.03
		Test Coupons	0.0012	0.00064	54	
	HIPS	Positive Controls	2.1	0.057	2.7	99.95 ± 0.01
		Test Coupons	0.00097	0.00026	26	
Dahlgren Decon – No Part A (No rinse)	ABS	Positive Controls	2.1	0.15	7.0	91.16 ± 0.98
		Test Coupons	0.19	0.016	8.5	
	Silicone	Positive Controls	2.1	0.039	1.9	84.74 ± 7.4
		Test Coupons	0.32	0.15	48	
	Gorilla Glass	Positive Controls	2.2	0.053	2.5	77.30 ± 15
		Test Coupons	0.49	0.31	64	
	HIPS	Positive Controls	2.1	0.081	3.8	67.46* ± 37
		Test Coupons	0.70*	0.79*	113*	
Dahlgren Decon – No Part A (No Rinse) (Retest)	ABS	Positive Controls	2.1	0.065	3.1	90.10 ± 4.3
		Test Coupons	0.21	0.092	44	
	Silicone	Positive Controls	2.2	0.15	6.8	89.71 ± 2.3
		Test Coupons	0.23	0.049	22	
	Gorilla Glass	Positive Controls	2.2	0.063	2.9	85.60 ± 2.4
		Test Coupons	0.31	0.050	16	
	HIPS	Positive Controls	2.1	0.11	5.5	80.97 ± 8.4
		Test Coupons	0.40	0.17	44	

*One data point (Replicate #3) for the HIPS material is a calculated outlier data point. For reporting purposes in this table, the data point was not removed (see explanation below).

The HIPS material calculations of average A-234 mass recovered, standard deviation, %RSD, and efficacy from the modified Dahlgren Decon™ test (Test #3) had a calculated outlier data

point for the third replicate sample. Using the Dixon test for outliers, this data point is considered an outlier with 90% confidence. The outlier data point could be a result of the "spitting" decontamination spray, which resulted in an uneven distribution of decontaminant onto the HIPS sample replicates. Figure 14 is a photo of the sample in question following the modified Dahlgren Decon™ spray. As a best-case scenario, if this data point is removed from the data set, the average A-234 mass recovered would be 0.14 ± 0.027, RSD of 20%, with a decontamination efficacy of 93.54%.

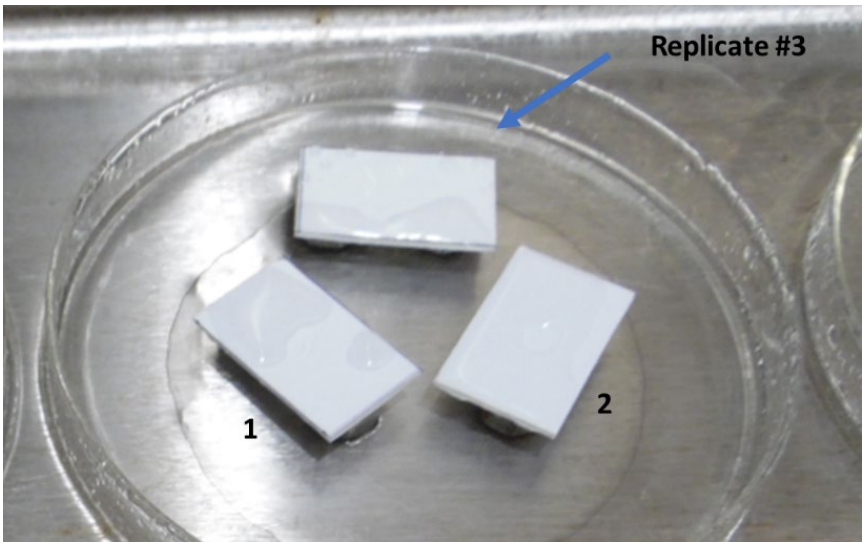

Figure 14. Photo of Potential HIPS outlier sample (Rep #3) following Decon Application

Overall, the Decon PLUS™ with the extended decontaminant dwell time (120 min) had a decontamination efficacy >99% for all four SE materials, but it did not increase the efficacy of previous tests run with Decon PLUS™ using a 60-min decontaminant dwell time. This would indicate that a longer dwell time for the Decon PLUS™ may provide no benefit to first responders in the field, at least in cases like the ones studied where the surface was comparatively clean, meaning it did not contain substances that might deplete the oxidant. The water-only decontaminant test (Test #1) had a relatively high efficacy range across the four materials (96.0 to 98.9%), but the analysis of the immediate rinsate samples (combined replicates) revealed that between 53% to 93% of the initial A-234 challenge (based on positive controls), remained in the rinsate. These results suggest that a water rinse only may be effective at physically removing A-234 from SE surfaces, which may be desirable for surfaces where any oxidant is undesirable. However, it will likely add complexity to full-scale operational remediation efforts because of potential hazards associated with residual A-234 remaining in the rinsates if they are not properly contained and treated. The Dahlgren Decon™ without Part A had poorer efficacy results for the SE materials than the water-only rinse (67% to 91%). These results may be due to the reasons discussed above relating to the Part A surfactant being removed or potential solubility issues with the A-234 and the Dahlgren Decon™ without the surfactant. The pH of the Dahlgren Decon™ without Part A was 7-8. The analysis of the immediate combined rinsate samples revealed that between 30% to 50% of the initial A-234 challenge (based on positive controls) was found in the rinsate, which may present challenges to full-scale operational remediation efforts, as discussed earlier.

Residual mass of A-234 in the combined rinsate samples for each material type is summarized in Figure 15. Average A-234 mass recoveries for the immediate rinsate samples and the rinsate samples analyzed after 24 hours are provided in Table 24.

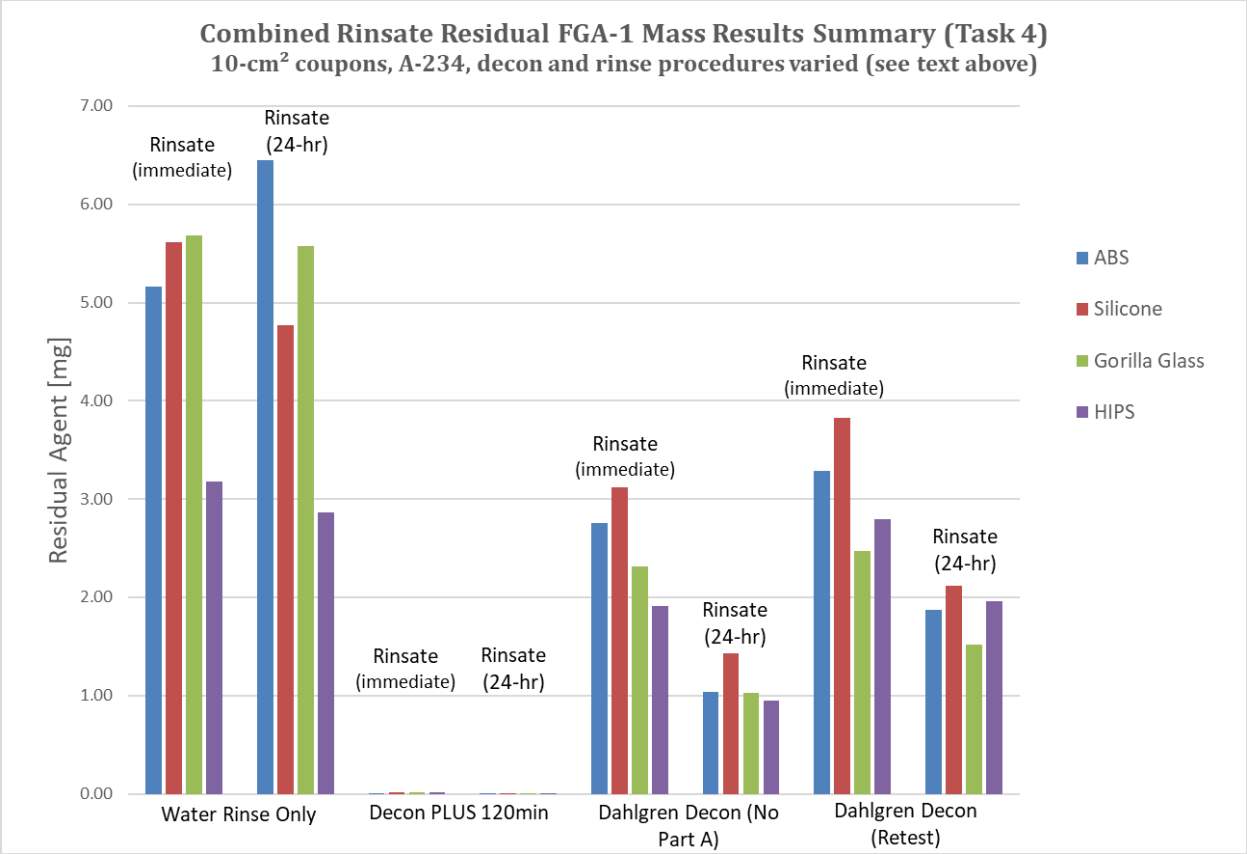

Figure 15. Residual A-234 in Rinsate Samples for Coupon Tests (Task 4)

Table 24. Residual A-234 in Rinsate Samples for Coupon Tests (Task 4)

Decon	Material	Rinsate (immediate) [mg]	Rinsate (24-hr) [mg]
Water Rinse Only – No Oxidant Applied	ABS	5.2	6.5
	Silicone	5.6	4.8
	Gorilla Glass	5.7	5.6
	HIPS	3.2	2.9
Decon PLUS™ – Decon Age Time = 120 min	ABS	0.0062	< 0.00020
	Silicone	0.016	0.0023
	Gorilla Glass	0.013	0.0015
	HIPS	0.016	0.0029
Dahlgren Decon – No Part A, No Rinse	ABS	2.8	1.0
	Silicone	3.1	1.4
	Gorilla Glass	2.3	1.0
	HIPS	1.9	0.95
Dahlgren Decon – No Part A, No Rinse (Retest)	ABS	3.3	1.9
	Silicone	3.8	2.1
	Gorilla Glass	2.5	1.5
	HIPS	2.8	2.0

3.2.4 Task 5 – Verification of Decon Efficacy Tests via Surface Sampling

Task 5 consisted of eight tests using large panels (302 cm²). No water rinsing was included following the decontamination spray for this task. Dahlgren Decon™ was eliminated from consideration due to its performance in previous tasks. The eight tests consisted of:

- Test 1: EasyDECON® DF200, HIPS panel
- Test 2: EasyDECON® DF200, silicone panel
- Test 3: Decon PLUS™, HIPS panel
- Test 4: Decon PLUS™, silicone panel
- Test 5: EasyDECON® DF200, HIPS panel, with blot sample
- Test 6: Decon PLUS™, HIPS panel, with blot sample
- Test 7: EasyDECON® DF200, HIPS panel, two decontaminant spray applications (60+60) with blot sample
- Test 8: Decon PLUS™, HIPS panel, two decontaminant spray applications (60+60) with blot sample

For reporting purposes, the masses detected in the blot sample and wipe sampler were added together for a total residual A-234 residual mass. Tests #7 and #8 added an additional decontaminant spray application to the panels. Following the 60-min dwell time for the first decontaminant spray application, the panels were tilted to allow the remaining decontaminant to

run off the panels, and then the second decontaminant spray application was completed (60-min dwell time). The samples were tilted again to allow decontaminant run-off, and then panels were sampled with the blot and wipe samplers which were quenched, extracted, and analyzed for residual A-234.

The spike control results of 100%, 105%, 104%, 96.7%, 104%, 99.8%, 95.7%, and 103% for tests 1 through 8, respectively, demonstrated that the contamination tool was performing within the established requirements. No A-234 was detected in any of the laboratory and procedural blank wipe, blot or rinsate samples for the eight (8) Task 5 tests. The decontamination efficacy results are summarized in Figure 16 and the detailed data are presented in Table 25. Efficacies were calculated by comparing the residual A-234 mass on the wipe samplers or the combined masses of the blot and wipe samplers against the A-234 mass from the associated positive controls.

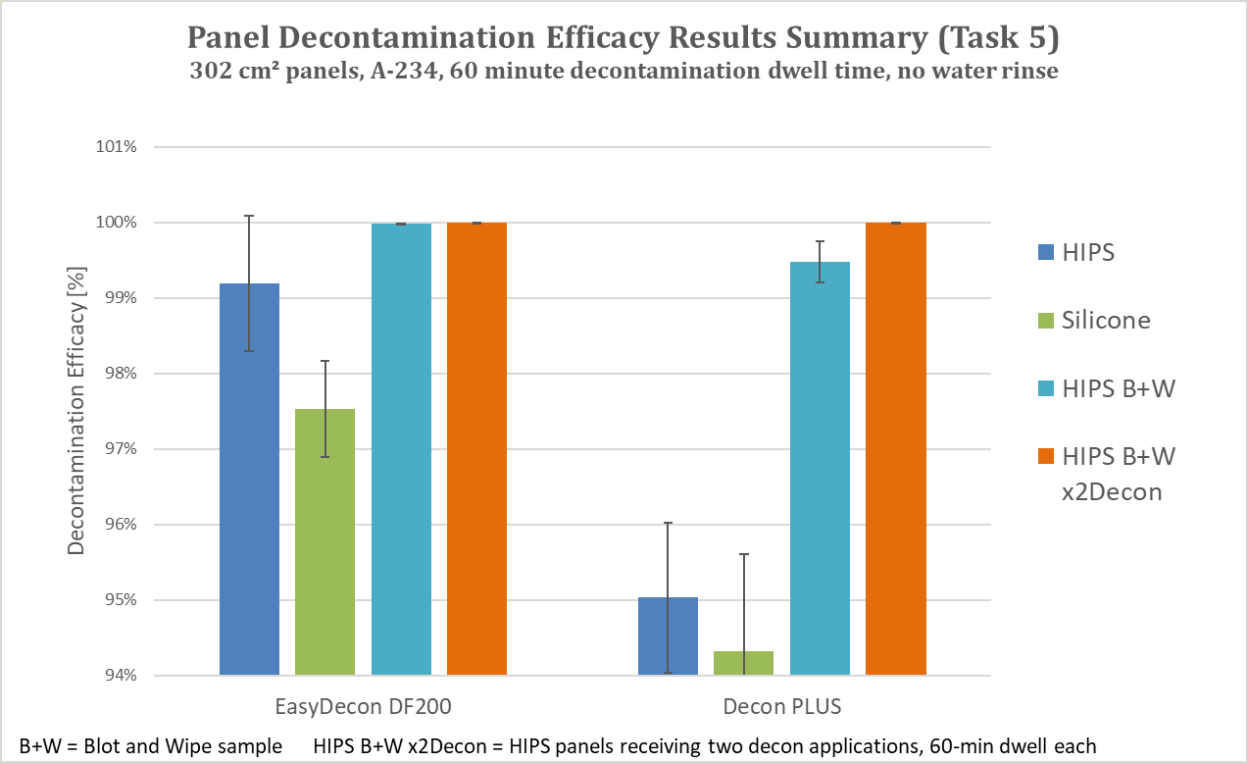

Figure 16. Decontamination Efficacy for Panel Tests (Task 5)

Table 25. Decontamination Efficacy for Panel Tests (Task 5)

Decon	Material	Sample Type	Mean [mg]	STDEV [mg]	RSD [%]	Efficacy [%]
EasyDECON DF200	HIPS	Positive Controls	55	4.2	7.6	99.19 ± 0.90
		Test Panels	0.45	0.49	110	
	Silicone	Positive Controls	54	1.1	2.1	97.53 ± 0.64
		Test Panels	1.3	0.34	26	
	HIPS B+W	Positive Controls	52	2.9	5.7	99.98 ± 0.01
		Test Panels (B+W)	0.0096	0.0036	38	
		Blot Sample	0.0081	0.0036	44	
		Wipe Sample	0.0014	0.00030	21	
	HIPS B+W x2Decon	Positive Controls	56	3.8	6.9	> 99.99 ± 0.0002
		Test Panels	< 0.0020	0	0	
		Blot Sample	<0.0010	0	0	
		Wipe Sample	<0.0010	0	0	
Decon PLUS™	HIPS	Positive Controls	55	2.8	5.2	95.03 ± 0.99
		Test Panels	2.7	0.53	19	
	Silicone	Positive Controls	47	3.8	8.2	94.32 ± 1.3
		Test Panels	2.7	0.56	21	
	HIPS B+W	Positive Controls	57	3.3	5.8	99.48 ± 0.27
		Test Panels	0.30	0.15	51	
		Blot Sample	0.11	0.082	73	
		Wipe Sample	0.19	0.072	38	
	HIPS B+W x2Decon	Positive Controls	53	3.0	5.7	> 99.99 ± 0.0002
		Test Panels	< 0.0020	0	0	
		Blot Sample	<0.0010	0	0	
		Wipe Sample	<0.0010	0	0	

B+W = Blot sample and wipe sample x2Decon = Two decontaminant sprays (60 min dwell time each)

Based on statistical tests (Section 2.6), the EasyDECON® DF200 had significantly lower mean residual A-234 mass on the HIPS and silicone panels when processed with one decontaminant application and wipe sampling only when compared to Decon PLUS™. Adding a blotting step prior to the wipe sampling significantly decreased the mean residual A-234 mass on the HIPS panels when Decon PLUS™ was used. Although the mean residual A-234 mass decreased with the addition of the blotting step for EasyDECON® DF200, it was within experimental error and was not significantly different from the wipe sampling only result. Adding a second decontaminant application decreased the mean residual A-234 mass for both EasyDECON® DF200 and Decon PLUS™ compared to using one decontaminant application with the blotting and wipe sampling process. However, based on experimental error and variance in data, only the EasyDECON® is considered a significantly lower result for residual A-234 mass.

Residual mass of A-234 in the combined rinsate samples for each material type is summarized in Figure 17. Average A-234 mass recoveries for the immediate rinsate samples and rinsate samples analyzed after 24 hours are provided in Table 26.

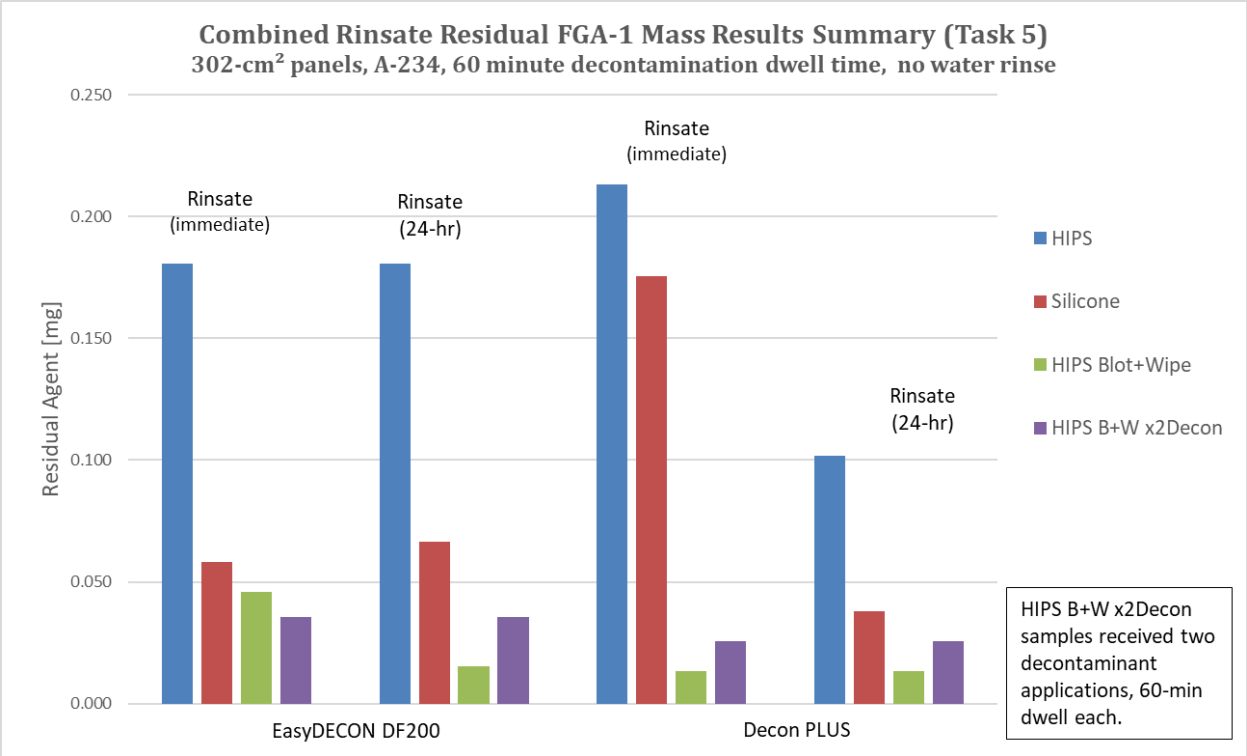

Figure 17. Residual A-234 in Rinsate Samples for Panel Tests (Task 5)

Table 26. Residual A-234 in Rinsate Samples for Coupon Tests (Task 5)

Decon	Material	Rinsate (immediate)	Rinsate (24-hr)
		[mg]	[mg]
EasyDECON DF200	HIPS	0.18	0.18
	Silicone	0.058	0.066
	HIPS Blot + Wipe	0.046	< 0.015
	HIPS B+W x2Decon	< 0.036	< 0.036
Decon PLUS™	HIPS	0.21	0.10
	Silicone	0.18	0.038
	HIPS Blot + Wipe	< 0.013	< 0.013
	HIPS B+W x2Decon	< 0.026	< 0.026

3.2.5 Task 6 – Decon Efficacy Tests with Sensitive Equipment

Task 6 consisted of two tests using SE items, namely iPhones and calculators. No water rinsing was done following the decontamination spray for this task.

- Test 1: Decon PLUS™, two SE items (5 replicates per item), two decontaminant spray applications (60+60) with blot sample
- Test 2: EasyDECON® DF200, two SE items (5 replicates per item), two decontaminant spray applications (60+60) with blot sample

The spike control results of 103% and 93.8% for Tests 1 and 2, respectively, demonstrated that the contamination tool was performing within the established requirements. No A-234 was detected in any laboratory or procedural blank (wipe and rinsate samples) for either test. The decontamination efficacy results are summarized in Figure 18, and the detailed data are presented in Table 27. Efficacies were calculated by comparing the residual A-234 mass of the blot and wipe samplers against the A-234 mass from the associated positive controls. For the calculators, the mean mass of the three positive control replicates that were contaminated on top of the buttons was used in the decontamination efficacy calculations.

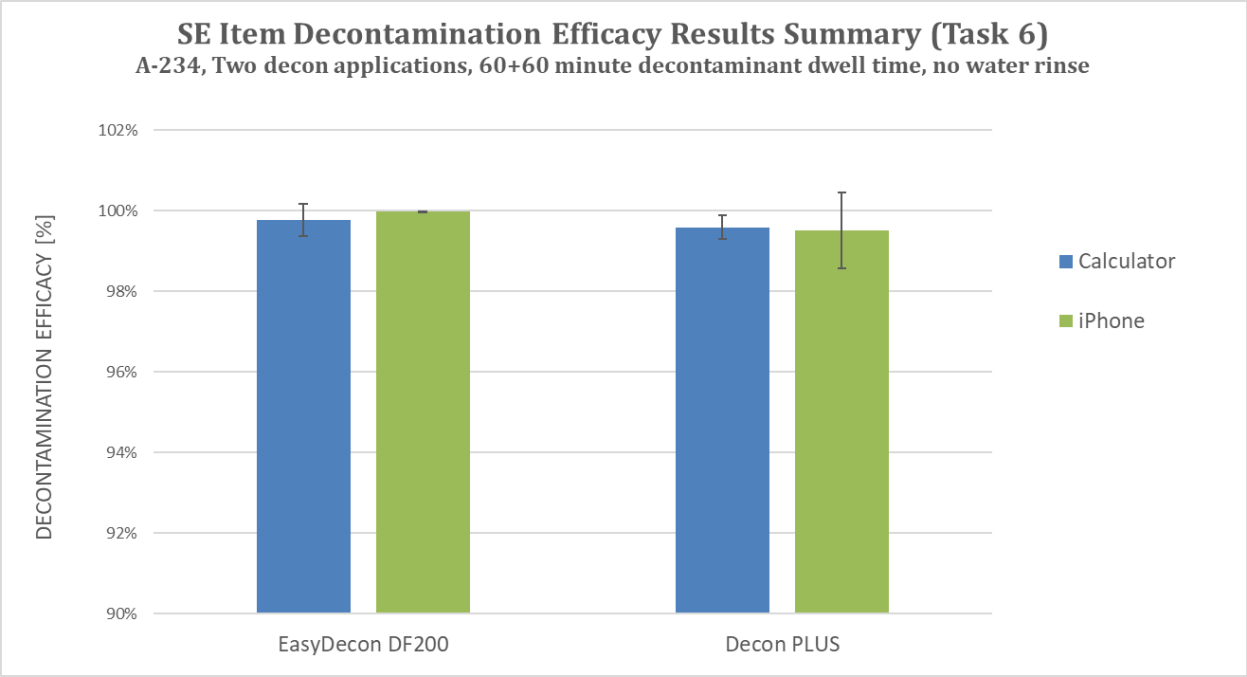

Figure 18. Decontamination Efficacy for SE Item Tests (Task 6)

Table 27. Decontamination Efficacy for SE Item Tests (Task 6)

Decon	Material	Sample Type	Mean [mg]	STDEV [mg]	RSD [%]	Efficacy [%]
EasyDECON DF200	SE Item 1 (iPhone)	Positive Controls	16	1.6	10	> 99.99 ± 0.001
		iPhones (B+W)	< 0.0020	0	0	
		Blot Sample	< 0.0010	0	0	
		Wipe Sample	< 0.0010	0	0	
	SE Item 2 (Calculator)	Positive Controls (top of buttons)	34	2.0	5.9	99.78 ± 0.41
		Positive Controls (recessed areas)	5.6	0.19	3.3	
		Calculators (B+W)	0.078	0.062	183	
		Blot Sample	0.014	0.0091	67	
		Wipe Sample	0.064	0.055	86	
Decon PLUS™	SE Item 1 (iPhone)	Positive Controls	17	1.5	8.7	99.51 ± 0.95
		iPhones* (B+W)	0.082	0.16	194	
		Blot Sample	0.077	0.15	198	
		Wipe Sample	0.0046	0.0072	155	
	SE Item 2 (Calculator)	Positive Controls (top of buttons)	33	0.44	1.3	99.60 ± 0.30
		Positive Controls (recessed areas)	13	6.4	49	
		Calculators (B+W)	0.13	0.10	75	
		Blot Sample	0.036	0.037	102	
		Wipe Sample	0.096	0.072	75	

* One of the iPhone replicates (#4) used in the calculations presented in the table is an outlier according to both Extreme Value (Dixon) and Discordance tests. For reporting purposes in this table, the data point was not removed.
B + W: blot and wipe

Both the EasyDECON® and Decon PLUS™ achieved decontamination efficacies greater than 99.5%.

There was a large difference in the positive control A-234 recovery between the calculators contaminated on top of the buttons (34.4 mg and 32.8 mg for Tests #1 and #2, respectively) and those contaminated in the button recesses (5.64 mg and 13.2 mg for Tests #1 and #2, respectively). The lower recovery from the recessed areas indicates that the wipe sampling method is unable to effectively collect the analyte from complex surfaces. This could cause false negative surface sampling results and highlights the need for a methodology that can assure decontamination of complex surfaces and/or provide more effective collection from complex surfaces, including recessed areas.

As noted in Table 27, the presented calculations for the Decon PLUS™ (Test #2) iPhone include a data point that is a calculated outlier at the 90% confidence level using the Dixon test. The outlier could be a result of an uneven distribution of decontaminant on the item surface. It was observed that the decontaminant tended to run off the glass surface of the iPhones or coalesce in

certain spots on the glass surface. Figure 19 is a photo of the sample in question following the two Decon PLUS™ spray applications. If this data point were removed from the data set, the average A-234 mass recovered would be 0.00261 ± 0.000822 mg, with an RSD of 31.5% and a decontamination efficacy of 99.98%. When comparing EasyDECON® and Decon PLUS™, statistical analysis confirms there is no significant difference in the reduction of A-234 from the surface of either the calculators or the iPhones.

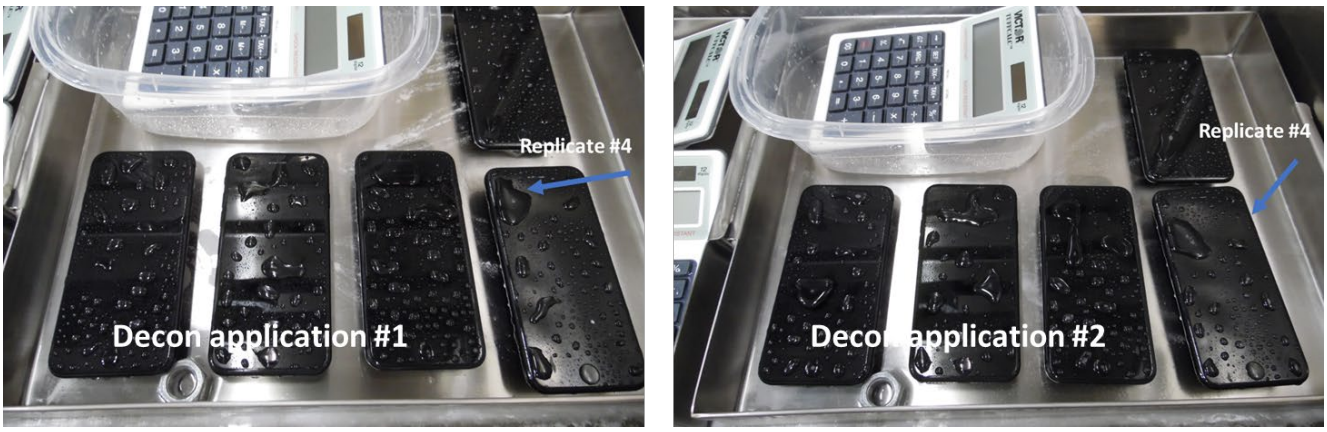

Figure 19. Photo of Potential iPhone Outlier Sample (Rep #4) following Decon Applications

Residual mass of A-234 in the combined rinsate samples for each SE item is summarized in Figure 20. Average A-234 mass recoveries for the immediate rinsate samples and rinsate samples analyzed after 24 hours are provided in Table 28.

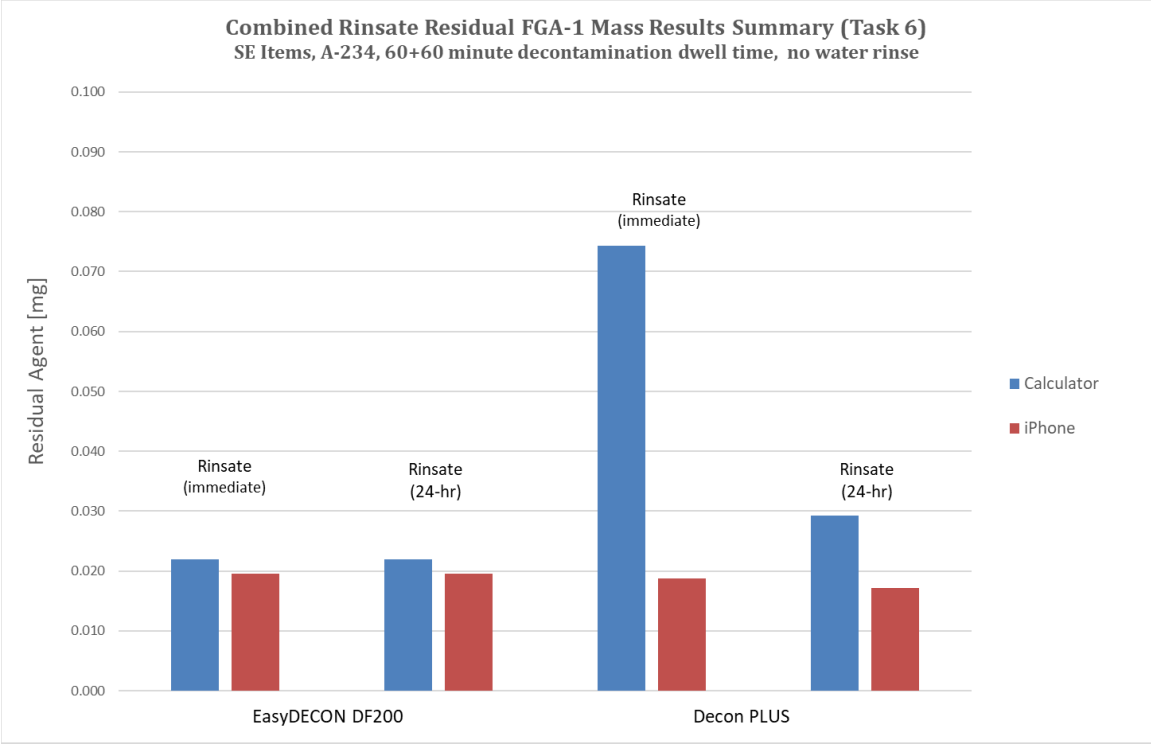

Figure 20. Residual A-234 in Rinsate Samples for SE Item Tests (Task 6)

Table 28. Residual A-234 in Rinsate Samples for SE Item Tests (Task 6)

Decon	Material	Rinsate (immediate)	Rinsate (24-hr)
		[mg]	[mg]
EasyDECON DF200	iPhone	< 0.020	< 0.020
	Calculator	0.022	< 0.022
Decon PLUS™	iPhone	0.019	< 0.017
	Calculator	0.074	< 0.029

Following the testing, each SE item (five sample replicates, one procedural and lab blank, and five positive control replicates) was tested to determine if the decontaminants had any negative effects on functionality. The results in Table 29 show a number of failures for the calculators. Many of the calculators that failed appeared to be constructed in a manner that the waterproofing was not secure over the display. All that passed appeared to have intact waterproofing. Note that the calculators are not claimed to be tested to any waterproof standard for electronics, whereas the iPhones are. This suggests that SE without tested waterproofing claims should be decontaminated cautiously with any liquid-based approach.

Table 29. SE Item Function Tests

Item	Type	Replicate	EasyDECON DF200	Decon PLUS™
			Function Test	Function Test
iPhone	Sample	R1	Pass	Pass
iPhone	Sample	R2	Pass	Pass
iPhone	Sample	R3	Pass	Pass
iPhone	Sample	R4	Pass	Pass
iPhone	Sample	R5	Pass	Pass
iPhone	Lab Blank	R1	Pass	Pass
iPhone	Procedural Blank	R1	Pass	Pass
iPhone	Positive Control	R1	Pass	Pass
iPhone	Positive Control	R2	Pass	Pass
iPhone	Positive Control	R3	Pass	Pass
iPhone	Positive Control	R4	Pass	Pass
iPhone	Positive Control	R5	Pass	Pass
Calculator	Sample	R1	Fail	Fail
Calculator	Sample	R2	Fail	Pass
Calculator	Sample	R3	Fail	Pass
Calculator	Sample	R4	Fail	Fail
Calculator	Sample	R5	Fail	Fail
Calculator	Lab Blank	R1	Pass	Pass

Item	Type	Replicate	EasyDECON DF200	Decon PLUS™
			Function Test	Function Test
Calculator	Procedural Blank	R1	Fail	Pass
Calculator	Positive Control	R1	Pass	Pass
Calculator	Positive Control	R2	Pass	Pass
Calculator	Positive Control	R3	Pass	Pass
Calculator	Positive Control	R4	Pass	Pass
Calculator	Positive Control	R5	Pass	Pass

QUALITY ASSURANCE/QUALITY CONTROL

4.1 Data Quality Indicators

Data quality indicators for this test program are outlined in Table 30. Meeting these data quality indicators limited the associated errors introduced into the reported results from this evaluation.

Table 30. Data Quality Indicators

Parameter	Measurement Method	Data Quality Indicators	Results
Temperature and RH	NIST-traceable thermometer/hygrometer	Current manufacturer calibration Test specifications: 18-24°C 30-70% RH	All temperature and RH measurements were within specification except: Task 4 Test #2: Humidity reached 72%, out of spec. for 40 min. Task 5 Test #5: Humidity reached 28%, out of spec. 20 min. Task 5 Test #7: Humidity reached 27% and temperature reached 17.5°C, out of spec. for 60 min.
Contamination Tool (syringe with dispenser)	NIST-traceable calibrated scale using distilled water. Mass must be within ±10% of expected mass	Once before use on first test, dispense known quantity. Found mass within ±10% of expected mass	Five gravimetric measurements were taken of 5x2µL drops of DI water Measurements of mass delivered were 98-99% of expected
Volume (mL)	Bottle dispenser for solvent	Prior to each experiment, dispense known volume into class A graduated cylinder. Volume must be within ±0.5 mL of expected volume	All checks of the bottle top dispensers prior to each experiment were within specifications
Volume (mL, µL)	Syringe and Electronic pipettors	Check the accuracy and repeatability one time before use by determining the mass delivered from known volume dispenses of DI water; found mass must be within ±10% of expected mass	All checks of pipettors prior to each experiment were within specifications
Decontaminant surface density (µL/cm²)	Decontamination Sprayer System	Each decontaminant technology was checked once prior to the start of testing. Decontaminant delivery was measured gravimetrically, using a NIST-traceable calibrated scale, over eight separate areas of the spray field, found mass must be within ±25% of target mass	Validation of three decontaminant sprays (Dahlgren Decon™, EasyDECON® DF200, and Decon PLUS™) were within target mass to achieve a coverage density of 60-100 µL/cm²
A-234 analysis and quantification	LC-MS/MS primary (P) and secondary (S) ion ratios will be monitored to ensure no interferences are present	The S/P ion ratios for all calibration standards and samples in an analytical sequence are checked to ensure ratios are within ±25% of the average ratio of calibration standards	All S/P ratios for all analyses were within specifications

Parameter	Measurement Method	Data Quality Indicators	Results
A-234 analysis and quantification	Calibration and Continuous Calibration Verification (CCV)	LC-MS/MS calibrations were completed at the start and end of each analytical sequence Calibration: R^2>0.990 and Δ% for any point is ±30% of nominal value (±50% at Method Response Limit [MRL]) CCV required for every 10 samples in an analytical sequence CCV: Calculated concentration must be ±30% of nominal value	Calibration curves and CCV's for all reported data are within specifications
Spike Control Samples	Extraction, LC-MS/MS	One set of three replicate spike controls run during each test Must be within 70 to 130% of target mass and <30% RSD	All spike controls for all tests were within specifications
Positive Control Samples	Extraction, LC-MS/MS	One set for each SE material type for each test Must be within 70 to 130% of contaminated amount based on spike control samples	All positive controls were within specification except for Task 6 Test #1 and Test #2 on the two calculator replicates contaminated in button recessed areas: Task 6 Test #1: 31.6% recovery Task 6 Test #2: 14.9% recovery
Laboratory and Procedural blanks	Extraction, LC-MS/MS	One for each SE material type for each test Target analyte response <0.5 MRL (equal to lowest calibration standard)	All laboratory and procedural blanks were within specification except: Task 4 Test #3: Procedural blank for ABS material = 0.00012 mg Task 4 Test #4: Procedural blank for ABS material = 0.00011 mg MDL = 0.0001 mg
Internal Standards	Extraction, LC-MS/MS	Internal standard added to extraction solvent at 0.1 ng/µL. Response must be within ±30% of the IS in the most recent CCV	IS response for all reported data were within the required specifications

4.2 Instrument Calibration

4.2.1 Calibration Schedule

Instruments and equipment used to execute this test program were maintained and operated according to Avarint's quality standards. All instruments were calibrated per manufacturer instructions, at a minimum annually, or were calibrated and/or checked for accuracy. The LC-MS/MS was calibrated as described in Section 4.2.2, and Table 31 provides calibration schedules for instruments that were used during the evaluation.

Table 31. Calibration Schedule

Equipment	Frequency
Calibrated pipettes	Calibration/accuracy verification was performed gravimetrically prior to each test using a NIST-traceable calibrated scale.
Model XS204 analytical balance	Calibration conducted annually by Precision Scale and Balance using NIST-traceable reference standards. Accuracy checked using NIST-traceable reference weights each day of use.
Calibrated Lascar EL-21CFR-2-LCD+ Hygrometer/Thermometer	Prior to the investigation by the manufacturer. Calibration did not expire before test was completed.
LC-MS/MS	Internal Mass Resolution and Calibration conducted quarterly and following any instrument maintenance. Calibrated prior to analysis of each set of samples (calibration curve) and calibration curve verification standards were analyzed after every ten samples (refer to Section 4.2.2).

4.2.2 LC-MS/MS Calibration

Calibration standards (from A-234 synthesized at Avarint, purity 99-100%), spanning the anticipated range of analysis, were created to generate calibration curves on the LC-MS/MS. Calibration standards were made fresh prior to each test day. A minimum of five calibration reference standards were analyzed at the beginning and end of each batch of samples, and the range of the calibration curve had to include the sample responses. Any sample response which exceeded the highest calibration standard was diluted and reanalyzed. Calibration curves were generated in Microsoft Excel® using a second-order polynomial fit. The correlation coefficient (R^2) was calculated from the regression fit of the calibration data and was required to be greater than 0.990. If a value of R^2 greater than 0.990 was not achieved, the LC-MS/MS instrument was evaluated to determine the root cause. Corrections were performed as needed, with a new calibration being performed and test samples re-analyzed (when necessary). When quantitated using the calibration curve, each calibration point was required to calculate within 70 to 130% (and 50-150% for lowest calibration standard) of its nominal value. Continuous calibration verification (CCV) standards (near the mid and low end of the calibration curve) were inserted into the analysis runs at least once for every 10 samples to identify potential calibration drift. The acceptance criteria for the CCVs were ±30% of the amount. Analytical results within the calibration range established for the instrument were reported in ng.

Solvent blank samples were included during the analytical runs to confirm that no A-234 carryover occurred. Solvent blank sample analysis results had to be < 0.5 MRL, which was set at the lowest calibration standard.

DISCUSSION/CONCLUSIONS

The purpose of this project was to evaluate the efficacy of commercial off the shelf (COTS) peroxy (i.e., activated hydrogen peroxide-based and/or peroxyacetic acid-based) decontamination technologies for decontamination of FGA on sensitive equipment materials.

The peroxy-based decontaminants that were investigated in this study include Dahlgren Decon™, Decon PLUS™, and EasyDECON® DF200. The Dahlgren Decon™ and Decon PLUS™ are both activated peroxyacetic acid technologies. The EasyDECON® DF200 is a hydrogen peroxide-based technology with an activator which leads to a peroxy acid. The four SE materials selected for this study were ABS, silicone, Gorilla Glass®, and HIPS. In addition, two types of sensitive equipment proxies (water resistant calculators and iPhones) were also included in the study. The FGA A-234 was applied to the surface test coupons having a surface area of 10 cm^2, test panels having a surface area of 302 cm^2, and full SE items as liquid droplets to achieve a target surface contamination density of 2 g/m^2.

Decontaminants were applied using a semi-automated spray system at a target application volume of 60 to 100 $\mu L/cm^2$. Following the specified decontaminant dwell periods, the test coupons, wipes and/or decontaminant overspray/rinsates were quenched and extracted in organic solvent and analyzed using LC-MS/MS to quantify the mass of A-234 remaining in the extracts. Decontamination efficacies were calculated by comparing the mean mass of A-234 remaining on the test samples to the mean mass of A-234 measured on the corresponding positive control samples.

Prior to conducting the decontamination efficacy tests, method demonstration/development studies were performed to demonstrate that the A-234 could be extracted at acceptable levels from the SE material substrates and the wipe sampler material. The results showed an extraction efficiency of > 92% for all four SE materials and the wipe sampler material, with one exception: the extraction efficiency of A-234 from the wipe sampler material was approximately 77% at the lowest contamination dose of 0.0004 g/m^2. This may reflect uncertainty associated with the quantitation limit.

Additional method demonstration/development studies were conducted to assess and optimize methods to quench the decontamination reactions within the extract matrices to prevent continued degradation of A-234. The quench methods investigated include 1) solvent extraction using isopropanol, 2) extraction in isopropanol with the addition of a 3M STS quenching solution, and 3) extraction in toluene with the addition of a 3M STS quenching solution. The final selected quenching methods were as follows:

- EasyDECON® DF200 and Decon PLUS™ used 3M STS quenchant and isopropanol as the extraction solvent for all four SE materials and the wipes.
- Dahlgren Decon™ required the addition of a water rinse prior to quenching and extraction. 3M STS quenchant and isopropanol were used for all four SE materials and the wipes.
- 3M STS quenchant and toluene extraction solvent were used for all three decontaminants for the rinsate samples.

The EasyDECON® DF200 and Decon PLUS™ decontaminants using a 60-min dwell time (Task 2) demonstrated a decontamination efficacy > 99.8% on three of the four SE material types (ABS, Gorilla Glass, and HIPS). The efficacies for silicone were slightly lower, at 99.5% for EasyDECON® DF200 and 99.1% for Decon PLUS™, and considered significantly different from the other three materials based on ANOVA and Tukey-HSD statistical tests. A small amount of residual A-234 was detected in the initial (immediate) rinsate samples for the EasyDECON® DF200 (0.00031 to 0.0007 mg), and no A-234 was detected in the 24-hr rinsate samples. No residual A-234 was detected in either the immediate or the 24-hr rinsate samples for the Decon PLUS™ with the exception of the silicone material, which had 0.0003 mg in the immediate rinsate. The Dahlgren Decon™ was not evaluated in this task because of the issues encountered with the quenching.

These same experiments were repeated using a water rinse (Task 3) following the decontamination step and before the sample extraction. Dahlgren Decon™ was included in this task. The EasyDECON® DF200 and Decon PLUS™ decontaminants using a 60-min dwell time and the water rinse demonstrated > 99.3% efficacy on all four SE material types. The Dahlgren Decon™ decontamination efficacy ranged from 91.4% to 97.1% on the SE material coupons. The mean residual A-234 on the silicone samples was significantly higher than the other three materials for EasyDECON® DF200 and Decon PLUS™, while there was no significant difference between materials with the Dahlgren Decon™.

Residual A-234 was detected in the Dahlgren Decon™ immediate rinsate samples, ranging from 4.5 to 6.3 mg, while < 0.0023 mg was found in the immediate rinsates from EasyDECON® DF200 and Decon PLUS™. For the 24-hour rinsate samples, no A-234 was detected in either of the EasyDECON® DF200 or Decon PLUS™ samples, while 3.9 to 4.6 mg of A-234 was detected in the Dahlgren Decon™ samples. The significant amounts of A-234 found in the Dahlgren Decon™ rinsate samples indicate that A-234 is physically being removed from the material surfaces but is not being effectively degraded. This will likely present challenges to full-scale operational remediation efforts because of potential hazards associated with residual A-234 remaining in the decontamination rinsates, if these rinsates are not appropriately managed.

The alternate decontamination approaches evaluated in Task 4 included 1) water rinse only (i.e., no oxidant applied to samples), 2) use of the Decon PLUS™ with a 120-min decontaminant dwell time, and 3) use of the Dahlgren Decon™ without the Part A surfactant (replacing it with de-ionized water).

Regarding the alternate approaches, Decon PLUS™ with a 120-min dwell time was the most efficacious with > 99.1% efficacy, followed by the water rinse only with > 95.9% efficacy. Although the Decon PLUS™ with a 120-min dwell time had a higher mean decontamination efficacy, based on experimental error and data variance, it is not significantly different from the water-only test. The Dahlgren Decon™ without the Part A surfactant performed the poorest with > 67.4% efficacy (> 80.8% for the retest); however, this decontaminant approach caused issues with the DSS that may have influenced the results as noted in Section 3.2.3.

Increasing the Decon PLUS™ dwell time from 60 to 120 min showed no statistically significant improvement in efficacy, indicating that the 60-min dwell time is sufficient and will provide the maximum efficacy under the conditions and parameters tested. Small quantities of A-234 were detected in the immediate (< 0.017 mg) and 24-hr (< 0.003 mg) rinsate samples across the four SE material types. As expected, rinsate samples for the water rinse only test and the Dahlgren

Decon™ test had significant levels of A-234 remaining, reinforcing the notion that physical removal of A-234 is occurring, but reactive decontamination is not.

Following Task 4, the better performing decontamination approaches were selected and evaluated at a larger scale on SE material panels in Task 5. Only the EasyDECON® DF200 and Decon PLUS™ decontaminants were evaluated because of the poor performance of Dahlgren Decon™, as well as the quenching and spray system issues that were encountered in the previous tasks. The Task 5 experiments conducted for the two decontaminants are listed here:

- HIPS, 60-min decontaminant dwell time, wipe sampling
- Silicone, 60-min decontaminant dwell time, wipe sampling
- HIPS, 60-min decontaminant dwell time, blotting and wipe sampling
- HIPS, two sequential decontaminant applications (each with a 60-min dwell time), blotting and wipe sampling

Silicone proved to be the most difficult material to decontaminate, with efficacies of 97.5% and 94.3% for EasyDECON® DF200 and Decon PLUS™, respectively. There was a statistical difference between the performance of the EasyDECON® DF200 and Decon PLUS™, with the EasyDECON® DF200 having higher decontamination efficacies for both the HIPS and silicone panels. For the decontaminant tests with two spray applications, both decontaminants demonstrated equivalent efficacies exceeding 99.99%. Statistical analysis indicates that incorporating a blotting step will significantly increase the overall efficacy on the HIPS material (by 4.5%) for the Decon PLUS™ decontaminant, but there was no difference with the EasyDECON® DF200.

The final testing task (Task 6) included evaluating SE items (calculators and iPhones) using EasyDECON® DF200 and Decon PLUS™ and a final set of parameters comprising two sequential decontaminant applications (each with a 60-min dwell time), blotting and wipe sampling. Based on statistical results, there was no significant difference in performance between EasyDECON® DF200 (99.8% and > 99.99% efficacy on the calculators and iPhones, respectively) and Decon PLUS™ (99.6% and 99.5% efficacy on the calculators and iPhones, respectively). Lastly, the SE item tests demonstrated that wipe sampling is likely not an effective method to collect A-234 from complex surfaces, as demonstrated by its inability to collect A-234 from the calculator button recesses of the positive control samples, where between 15% to 32% of the initial contamination was collected on the wipe sampler (compared to 79% to 91% of the initial contamination mass collected for the positive controls contaminated on the tops of the buttons). As such, caution should be employed by first responders when using this method to analyze complex surfaces for residual chemicals, and alternate methodologies should be developed and deployed in an operational environment.

REFERENCES

[1] See https://www.gov.uk/government/news/novichok-nerve-agent-use-in-salisbury-uk-government-response. Last accessed August 29, 2022

[2] See https://www.gov.uk/government/news/clean-up-work-completed-in-salisbury-as-area-continues-recovery. Last accessed August 29, 2022

[3] Oudejans, L. and D. See. 2019. "Efficacy and Compatibility of Decontamination Options for Sensitive Equipment-Related Materials Contaminated with Persistent Chemical Warfare Agents." U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-19/075.

Attachment A – Environmental Data

Table A1. Environmental Data

Task	Test #	Temperature °C		RH%		Deviation
		Low	High	Low	High	
2	1	21.5	22	56.5	59	
2	2	21.5	21.5	51	54.5	
3	1	21.5	22	55	60	
3	2	21.5	21.5	52.5	67	
3	3	21	21.5	47.5	51	
3	4	21.5	22	55.5	68.5	
4	1	21	22	45	65	
4	2	21.5	24	56	72	Out of spec. for 40 min.
4	3	21	21.5	40	43.5	
4	4	21	22	37	47	
5	1	21	21.5	35	37	
5	2	19	19.5	37.5	41	
5	3	18	20.5	37	39	
5	4	19	19.5	38.5	40.5	
5	5	18	19.5	28	34.5	Out of spec. for 20 min.
5	6	18	19.5	33.5	42	
5	7	17.5	19.5	27	36	Out of spec for 60 min.
5	8	19.5	23.5	32.5	53.5	
6	1	21	22	41	56.5	
6	2	19	20	36.5	64.5	

Attachment B – Spray Characterization Data

Table B1. Decontaminant Spray Characterization, EasyDECON® DF200

Petri Dish	Initial Wt.	Final Wt.	Wt. Δ	Volume	Volume	Dish Area	Volume
	g	g	g	mL	µL	cm²	µL/cm²
1	69.08	82.74	13.66	12.53	12,532	149.5	83.8
2	74.93	88.47	13.54	12.42	12,422	149.5	83.1
3	77.44	91.22	13.78	12.64	12,642	149.5	84.6
4	79.32	93.18	13.86	12.72	12,716	149.5	85.1
5	77.91	91.57	13.66	12.53	12,532	149.5	83.8
6	69.18	82.49	13.31	12.21	12,211	149.5	81.7
7	79.99	93.63	13.64	12.51	12,514	149.5	83.7
8	84.12	98.1	13.98	12.83	12,826	149.5	85.8
						Mean	83.9
						STD	1.17
						%RSD	1.49

Table B2. Decontaminant Spray Characterization, Decon PLUS™

Petri Dish	Initial Wt.	Final Wt.	Wt. Δ	Volume	Volume	Dish Area	Volume
	g	g	g	mL	µL	cm²	µL/cm²
1	74.93	89.65	14.72	14.02	14,019	149.5	93.8
2	79.33	94.05	14.72	14.02	14,019	149.5	93.8
3	69.07	83.17	14.10	13.43	13,429	149.5	89.8
4	77.44	92.21	14.77	14.07	14,067	149.5	94.1
5	69.18	83.52	14.34	13.66	13,657	149.5	91.4
6	84.11	98.08	13.97	13.30	13,305	149.5	89.0
7	79.98	94.42	14.44	13.75	13,752	149.5	92.0
8	77.93	92.69	14.76	14.06	14,057	149.5	94.0
						Mean	92.2
						STD	2.02
						%RSD	2.19

Table B3. Decontaminant Spray Characterization, Dahlgren Decon™

Petri Dish	Initial Wt.	Final Wt.	Wt. Δ	Volume	Volume	Dish Area	Volume
	g	g	g	mL	µL	cm²	µL/cm²
1	69.09	82.65	13.56	12.44	12,440	149.5	83.2
2	74.92	88.56	13.64	12.51	12,514	149.5	83.7
3	77.44	90.86	13.42	12.31	12,312	149.5	82.4
4	79.33	92.70	13.37	12.27	12,266	149.5	82.0
5	77.93	90.7	12.77	11.72	11,716	149.5	78.4
6	69.18	82.42	13.24	12.15	12,147	149.5	81.2
7	79.99	93.07	13.08	12.00	12,000	149.5	80.3
8	84.13	97.21	13.08	12.00	12,000	149.5	80.3
						Mean	81.4
						STD	1.76
						%RSD	2.16

Attachment C – Statistical Analysis Results

ANOVA Results

The statistical results for the ANOVA and Tukey-HSD comparisons are presented in Tables C1 through C13. Details of each statistical comparison are described in the following sections.

Group 1 Material Effect Results (Small Coupons)

Material Effect Results no Rinse (Small Coupons)

There was a significant difference in A-234 mass recovery, with recovery from silicone material being significantly higher than the other three materials (ABS, Gorilla Glass®, and HIPS) for a given decontaminant (Decon PLUS™ and EasyDECON® DF200). The mean mass recoveries for ABS, Gorilla Glass®, and HIPS were not significantly different from each other.

Table C1. ANOVA Results for Decon PLUS™ with No Rinse at 60min (Test Samples)

Decontaminant	Material	Decontaminant Dwell Time (min)	Q value	Critical Range	Mean Mass Recovery (mg)	Tukey-HSD Result
Decon PLUS™	ABS	60	4.53	0.00767	0.00044	SD ABS < Silicone SD Gorilla Glass < Silicone SD HIPS < Silicone ND ABS & Gorilla Glass ND ABS & HIPS ND Gorilla Glass & HIPS
	Silicone	60	4.53	0.00767	0.020	
	Gorilla Glass	60	4.53	0.00767	0.00018	
	HIPS	60	4.53	0.00767	0.00039	
Significant difference when absolute difference of means between materials is above critical range. ND = No significant difference SD = Significant difference						

Table C2. ANOVA Results for EasyDECON® DF200 with No Rinse at 60min (Test Samples)

Decontaminant	Material	Decontaminant Dwell Time (min)	Q value	Critical Range	Mean Mass Recovery (mg)	Tukey-HSD Result
EasyDECON® DF200	ABS	60	4.53	0.00214	0.00040	SD ABS < Silicone SD Gorilla Glass < Silicone SD HIPS < Silicone ND ABS & Gorilla Glass ND ABS & HIPS ND Gorilla Glass & HIPS
	Silicone	60	4.53	0.00214	0.010	
	Gorilla Glass	60	4.53	0.00214	0.00023	
	HIPS	60	4.53	0.00214	0.0013	
Significant difference when absolute difference of means between materials is above critical range. ND = No significant difference SD = Significant difference						

Material Effect Results with Rinse (Small Coupons)

There was a significant difference in A-234 mass recovery, recovery from with silicone material being significantly higher than the other three materials (ABS, Gorilla Glass®, and HIPS) when considered with the decontaminants Decon PLUS™ and EasyDECON® DF200. The mean mass recoveries for ABS, Gorilla Glass®, and HIPS with these two decontaminants were not significantly different from each other. For the Dahlgren Decon™, there was no significant differences in the mean A-234 mass recoveries for the four SE materials.

Table C3. ANOVA Results for Decon PLUS™ with Water Rinse at 60min (Test Samples)

Decontaminant	Material	Decontaminant Dwell Time (min)	Q value	Critical Range	Mean Mass Recovery (mg)	Tukey-HSD Result
Decon PLUS™	ABS	60	4.53	0.00316	0.00013	SD ABS < Silicone SD Gorilla Glass < Silicone SD HIPS < Silicone ND ABS & Gorilla Glass ND ABS & HIPS ND Gorilla Glass & HIPS
	Silicone	60	4.53	0.00316	0.014	
	Gorilla Glass	60	4.53	0.00316	0.00015	
	HIPS	60	4.53	0.00316	0.00075	
Significant difference when absolute difference of means between materials is above critical range. ND = No significant difference SD = Significant difference						

Table C4. ANOVA Results for EasyDECON® DF200 with Water Rinse at 60min (Test Samples)

Decontaminant	Material	Decontaminant Dwell Time (min)	Q value	Critical Range	Mean Mass Recovery (mg)	Tukey-HSD Result
EasyDECON® DF200	ABS	60	4.53	0.00730	0.00025	SD ABS < Silicone SD Gorilla Glass < Silicone SD HIPS < Silicone ND ABS & Gorilla Glass ND ABS & HIPS ND Gorilla Glass & HIPS
	Silicone	60	4.53	0.00730	0.014	
	Gorilla Glass	60	4.53	0.00730	0.00011	
	HIPS	60	4.53	0.00730	0.00016	
Significant difference when absolute difference of means between materials is above critical range. ND = No significant difference SD = Significant difference						

Table C5. ANOVA Results for Dahlgren Decon™ with Water Rinse at 60min (Test Samples)

Decontaminant	Material	Decontaminant Dwell Time (min)	Q value	Critical Range	Mean Mass Recovery (mg)	Tukey-HSD Result
Dahlgren Decon™	ABS	60	4.53	0.149	0.060	ND No significant differences
	Silicone	60	4.53	0.149	0.069	
	Gorilla Glass	60	4.53	0.149	0.079	
	HIPS	60	4.53	0.149	0.18	

Significant difference when absolute difference of means between materials is above critical range. ND = No significant difference

SD = Significant difference

Group 2 Decontamination Process Effect Results (Small Coupons)

Decontamination Process Effect Results on ABS (Small Coupons)

There was a significant difference in A-234 mass recovery from ABS material, with the recovery from Dahlgren Decon™ with water rinse 60-min process being significantly higher than the Decon PLUS™ and EasyDECON® DF200 60-min processes (both with a water rinse and without). The recovery from Decon PLUS™ with water rinse 120-min process was also significantly lower than the Dahlgren Decon™ with water rinse 60-min process. It should be noted, based on statistical analysis, that none of the decontamination processes were statistically different from using water only as a decontaminant.

Table C6. ANOVA Results for ABS Material Across Decontamination Processes

Material	Decontaminant	Decontaminant Dwell Time (min)	Q value	Critical Range	Mean Mass Recovery (mg)	Tukey-HSD Result
ABS	Decon PLUS™ no rinse	60	4.83	0.0553	0.00044	SD DP no rinse < DD w/rinse SD ED no rinse < DD w/rinse SD DP w/rinse < DD w/rinse SD ED w/rinse < DD w/rinse SD DP 120min < DD w/rinse ND DP no rinse & ED no rinse ND DP no rinse & DP w/rinse ND DP no rinse & ED w/rinse ND DP no rinse & Water ND DP no rinse & DP 120min ND ED no rinse & DP w/rinse ND ED no rinse & ED w/rinse ND ED no rinse &Water ND ED no rinse & DP 120min ND DP w/rinse & ED w/rinse ND DP w/rinse & Water ND DP w/rinse & DP 120min ND ED w/rinse & Water ND ED w/rinse & DP 120min ND DD w/rinse & Water ND Water & DP 120min
	EasyDECON® DF200 no rinse	60	4.83	0.0553	0.00040	
	Decon PLUS™ w/rinse	60	4.83	0.0553	0.00013	
	EasyDECON® DF200 w/rinse	60	4.83	0.0553	0.0025	
	Dahlgren Decon™ w/rinse	60	4.83	0.0553	0.060	
	Water (no oxidant)	60	4.83	0.0553	0.023	
	Decon PLUS™ w/rinse	120	4.83	0.0553	0.00082	

Significant difference when absolute difference of means between materials is above critical range. ND = No significant difference
SD = Significant difference DP=Decon PLUS™ ED=EasyDECON DD=Dahlgren Decon

Decontamination Process Effect Results on Silicone (Small Coupons)

There was a significant difference in A-234 mass recovery from ABS material, with the recovery from Dahlgren Decon™ with water rinse 60-min process being significantly higher than the Decon PLUS™ and EasyDECON® DF200 60-min processes (both with a water rinse and without). The recovery Decon PLUS™ with water rinse 120-min process was also significantly lower than the recovery from Dahlgren Decon™ with water rinse 60-min process. This same correlation is found with the mean A-234 recovery for the water only process being significantly

higher than the Decon PLUS™ and EasyDECON® DF200 60-min processes (both with a water rinse and without) and the Decon PLUS™ with water rinse 120-min process.

Table C7. ANOVA Results for Silicone Material Across Decontamination Processes

Material	Decontaminant	Decontaminant Dwell Time (min)	Q value	Critical Range	Mean Mass Recovery (mg)	Tukey-HSD Result
Silicone	Decon PLUS™ no rinse	60	4.83	0.0304	0.020	SD DP no rinse < DD w/rinse SD DP no rinse < Water SD ED no rinse < DD w/rinse SD ED no rinse < Water SD DP w/rinse < DD w/rinse SD DP w/rinse < Water SD ED w/rinse < DD w/rinse SD ED w/rinse < Water SD DP 120min < DD w/rinse SD DP 120min < Water ND DP no rinse & ED no rinse ND DP no rinse & DP w/rinse ND DP no rinse & ED w/rinse ND DP no rinse & DP 120min ND ED no rinse & DP w/rinse ND ED no rinse & ED w/rinse ND ED no rinse & DP 120min ND DP w/rinse & ED w/rinse ND DP w/rinse & DP 120min ND ED w/rinse & DP 120min ND DD w/rinse & Water
	EasyDECON® DF200 no rinse	60	4.83	0.0304	0.010	
	Decon PLUS™ w/rinse	60	4.83	0.0304	0.014	
	EasyDECON® DF200 w/rinse	60	4.83	0.0304	0.014	
	Dahlgren Decon™ w/rinse	60	4.83	0.0304	0.069	
	Water (no oxidant)	60	4.83	0.0304	0.081	
	Decon PLUS™ w/rinse	120	4.83	0.0304	0.018	

Significant difference when absolute difference of means between materials is above critical range. ND = No significant difference

SD = Significant difference DP=Decon PLUS™ ED=EasyDECON DD=Dahlgren Decon

Decontamination Process Effect Results on Gorilla Glass® (Small Coupons)

There was no significant difference in the mean mass recoveries between any of the decontamination processes with Gorilla Glass®.

Table C8. ANOVA Results for Gorilla Glass® Material Across Decontamination Processes

Material	Decontaminant	Decontaminant Dwell Time (min)	Q value	Critical Range	Mean Mass Recovery (mg)	Tukey-HSD Result
Gorilla Glass®	Decon PLUS™ no rinse	60	4.83	0.0804	0.00018	ND No significant difference
	EasyDECON® DF200 no rinse	60	4.83	0.0804	0.00023	
	Decon PLUS™ w/rinse	60	4.83	0.0804	0.00015	
	EasyDECON® DF200 w/rinse	60	4.83	0.0804	0.00011	
	Dahlgren Decon™ w/rinse	60	4.83	0.0804	0.079	
	Water (no oxidant)	60	4.83	0.0804	0.051	
	Decon PLUS™ w/rinse	120	4.83	0.0804	0.0012	

Significant difference when absolute difference of means between materials is above critical range. ND = No significant difference

SD = Significant difference DP=Decon PLUS™ ED=EasyDECON DD=Dahlgren Decon

Decontamination Process Effect Results on HIPS (Small Coupons)

There was a significant difference in A-234 mass recovery from HIPS material, with the Dahlgren Decon™ with water rinse 60-min process being significantly higher than the Decon PLUS™ and EasyDECON® DF200 60-min processes (both with a water rinse and without). The recovery from Decon PLUS™ with water rinse 120-min process was also significantly lower than the Dahlgren Decon™ with water rinse 60-min process. This same correlation is found with the mean A-234 recovery for the water only process and Decon PLUS™ with water rinse 120-min process being significantly lower than the Dahlgren Decon™ with water rinse 60-min process. It should be noted, based on statistical analysis, that none of the decontamination processes were significantly better than using water only as a decontaminant.

Table C9. ANOVA Results for HIPS Material Across Decontamination Processes

Material	Decontaminant	Decontaminant Dwell Time (min)	Q value	Critical Range	Mean Mass Recovery (mg)	Tukey-HSD Result
HIPS	Decon PLUS™ no rinse	60	4.83	0.0857	0.00039	SD DP no rinse < DD w/rinse SD ED no rinse < DD w/rinse SD DP w/rinse < DD w/rinse SD ED w/rinse < DD w/rinse SD Water < DD w/rinse SD DP 120min < DD w/rinse ND DP no rinse & ED no rinse ND DP no rinse & DP w/rinse ND DP no rinse & ED w/rinse ND DP no rinse & DP 120min ND ED no rinse & DP w/rinse ND ED no rinse & ED w/rinse ND ED no rinse & DP 120min ND DP w/rinse & ED w/rinse ND DP w/rinse & DP 120min ND ED w/rinse & DP 120min ND DP no rinse & Water ND ED no rinse & Water ND DP w/rinse & Water ND ED w/rinse & Water ND DP 120min & Water
	EasyDECON® DF200 no rinse	60	4.83	0.0857	0.0013	
	Decon PLUS™ w/rinse	60	4.83	0.0857	0.00075	
	EasyDECON® DF200 w/rinse	60	4.83	0.0857	0.00016	
	Dahlgren Decon™ w/rinse	60	4.83	0.0857	0.18	
	Water (no oxidant)	60	4.83	0.0857	0.044	
	Decon PLUS™ w/rinse	120	4.83	0.0857	0.00097	

Significant difference when absolute difference of means between materials is above critical range. ND = No significant difference

SD = Significant difference DP = Decon PLUS™ ED = EasyDECON DD = Dahlgren Decon

Decontamination Period Effect Results (Small Coupons)

There was no significant difference in A-234 mass recovery from the four SE materials when comparing the recovery from Decon PLUS™ with water rinse 60-min process and the Decon PLUS™ with water rinse 120-min process. Hence the extended decontaminant dwell time for the Decon PLUS™ had no significant impact on the efficacy for the four SE materials.

Table C10. ANOVA Results for Decon Plus™ at 60- and 120-min Dwell Times

Material	Decontaminant	Decontaminant Dwell Time (min)	Mean Mass Recovery (mg)	p-Value	ANOVA Result
ABS	Decon PLUS™ w/rinse	60	0.00013	0.252	No significant difference
	Decon PLUS™ w/rinse	120	0.00082		
Silicone	Decon PLUS™ w/rinse	60	0.014	0.229	No significant difference
	Decon PLUS™ w/rinse	120	0.018		
Gorilla Glass®	Decon PLUS™ w/rinse	60	0.00015	0.0846	No significant difference
	Decon PLUS™ w/rinse	120	0.0012		
HIPS	Decon PLUS™ w/rinse	60	0.00075	0.662	No significant difference
	Decon PLUS™ w/rinse	120	0.00097		

Significant difference when absolute difference of means between materials is above critical range. ND = No significant difference

SD = Significant difference DP = Decon PLUS™ ED = EasyDECON DD = Dahlgren Decon

Group 3 Material Effect Results (Large Panels)

There was a significant difference in A-234 mass recovery from the HIPS and silicone large panels when comparing the Decon PLUS™ 60-min process and the EasyDECON® DF200 60-min process. The recovery from EasyDECON® DF200 60-min process was significantly lower than the Decon PLUS™ 60-min process for both the HIPS and silicone large panels.

Table C11. ANOVA Results for Decontaminants and Varying SE Panels

Material	Decontaminant	Decontaminant Dwell Time (min)	Mean Mass Recovery (mg)	p-Value	ANOVA Result
HIPS panel	Decon PLUS™	60	2.7	0.01	SD ED < DP
	EasyDECON® DF200	60	0.45		
Silicone panel	Decon PLUS™	60	2.7	0.048	SD ED < DP
	EasyDECON® DF200	60	1.34		

Significant difference when absolute difference of means between materials is above critical range. ND = No significant difference

SD = Significant difference DP = Decon PLUS™ ED = EasyDECON DD = Dahlgren Decon

Group 4 Decontamination Process Effect Results (Large Panels)

There was no significant difference in A-234 mean mass recovery with EasyDECON® DF200 when comparing the blot + wipe sampling process with wipe sampling only. There was also no significant difference in the recoveries between Decon PLUS™ when comparing the blot + wipe sampling process with one decon application to the two decon application process with the blot + wipe sampling process with two applications. A significant difference in A-234 mass recovery was seen, with the Decon PLUS™ blot + wipe process being significantly lower than with wipe sampling only. There was also a significant difference in recoveries using EasyDECON® DF200 and the blot + wipe sampling process, with recoveries for the double decontaminant applications process being significantly lower than when using one decontaminant application.

Table C12. ANOVA Results for Decontaminants and Varying Processes on HIPS Panels

Decontaminant	Material	Decontaminant Dwell Time (min)	Sampling Process	Mean Mass Recovery (mg)	p-Value	ANOVA Result
EasyDECON® DF200	HIPS panel	60	Wipe	0.45	0.28	ND No significant difference
	HIPS panel	60	Blot + Wipe	0.0096		
Decon PLUS™	HIPS panel	60	Wipe	2.7	0.003	SD DP B+W < DP
	HIPS panel	60	Blot + Wipe	0.30		
EasyDECON® DF200	HIPS panel	60 + 60	x2 Decon Blot + Wipe	0.002	0.040	SD EDx2Decon < ED B+W
	HIPS panel	60	Blot + Wipe	0.0096		
Decon PLUS™	HIPS panel	60 + 60	x2 Decon Blot + Wipe	0.002	0.052	ND No significant difference
	HIPS panel	60	Blot + Wipe	0.30		

Significant difference when absolute difference of means between materials is above critical range. ND = No significant difference
SD = Significant difference DP = Decon PLUS™ ED = EasyDECON DD = Dahlgren Decon B+W = Blot + Wipe sampling
60+60 = Two decontaminant applications (60 min each)

Group 5 Decontaminant Effect Results (SE Items)

There was no significant difference in the A-234 mean mass recoveries between EasyDECON® DF200 and Decon PLUS™ when decontaminating SE items (calculators and iPhones) using two decontaminant applications with the blot + wipe sampling process.

Table C13. ANOVA Results for Decontaminants and SE Items

SE Item	Decontaminant	Decontaminant Dwell Time (min)	Sampling Process	Mean Mass Recovery (mg)	p-Value	ANOVA Result
iPhones	EasyDECON® DF200	60 + 60	Blot + Wipe	0.082	0.34	ND No significant difference
	Decon PLUS™	60 + 60	Blot + Wipe	0.002		
Calculators	EasyDECON® DF200	60 + 60	Blot + Wipe	0.077	0.37	ND No significant difference
	Decon PLUS™	60 + 60	Blot + Wipe	0.13		

Significant difference when absolute difference of means between materials is above critical range. ND = No significant difference

SD = Significant difference DP = Decon PLUS™ ED = EasyDECON DD = Dahlgren Decon B+W = Blot + Wipe sampling

60+60 = Two decontaminant applications (60 min each)

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